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ANALOGS OF BIOACTIVE ARYLAKYLAMINES: NEUROCHEMICAL AND NEUROPHARMACOLOGICAL STUDIES IN THE RAT

by

©

TADIMETI SEETAPATI RAO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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AND FACULTY OF MEDICINE (PSYCHIATRY)

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SPRING, 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ANALOGS OF BIOACTIVE ARYLALKYLAMINES: NEUROCHEMICAL AND NEUROPHARMACOLOGICAL STUDIES IN THE RAT submitted by TADIMETI S. RAO in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Pharmaceutical Sciences (Neurochemistry).

Glen B. Baker
Supervisor

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External Examiner

Date November 21, 1986

Dedication

to my family

ABSTRACT

A major portion of the project dealt with the testing of N-alkylated analogs of 2-phenylethylamine (PEA) as possible 'prodrugs' to produce in brain selective (without elevating levels of other biogenic amines) increases in concentrations of PEA, an amine which has been implicated in the etiology of depressive disorders. Such prodrugs, by producing elevated and relatively sustained levels of PEA in brain, would be useful tools in future studies on the role of PEA in brain function and in antidepressant drug action. Analogs tested in a rat model included N-2-(cyanoethyl)-PEA (CEPEA), N-3-(chloropropyl)-PEA (CPPEA), N-propargyl-PEA (PGPEA), and N,N-dipropargyl-PEA (DPGPEA). Time studies were carried out on levels of PEA and the N-alkylated analogs in brain (and also in liver and blood) following intraperitoneal injection of the analogs. All four analogs produced elevated, sustained PEA levels in brain, but their relative potency in elevating PEA was PGPEA>DPGPEA>CEPEA>CPPEA. Neurochemical effects of the elevated PEA levels combined with the prodrug levels in the brain were studied by measuring brain levels of putative neurotransmitter amines and their acid metabolites. None of the prodrugs caused marked, sustained elevations of these biogenic amines, and in some cases there were actually depletions.

A preliminary study was conducted with N-cyanoethyltryptamine (CET) as a possible prodrug of tryptamine, but this analog was not a promising prodrug.

Also investigated were N-alkylated and phenyl ring-substituted analogs of tranylcypromine (TCP), an antidepressant similar structurally

to PEA. Several N-alkylated analogs studied were equipotent to, or stronger than, TCP at inhibiting MAO *in vitro*, and 4-fluoro-TCP (FTCP) was studied in detail *in vivo*. A preliminary study was also carried out *in vivo* with N-propargyl-TCP. 4-Fluoro-TCP had more favourable pharmacokinetic properties than TCP and caused a different pattern of elevation of brain amines. N-Propargyl-TCP was not a particularly effective prodrug of TCP.

To conduct the studies described above, sensitive and selective analytical procedures for PEA, T, TCP and the analogs were developed. Extractive derivatization procedures utilizing either pentafluorobenzoyl chloride or pentafluorobenzenesulfonyl chloride were followed by analysis using electron-capture gas chromatography.

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TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

A. Prodrugs 1

B. Neuroleptics 5

B.1 Phenothiazines 5

B.2 Other neuroleptics 6

C. Tricyclic Antidepressants 7

D. Catecholamines 8

E. γ-Aminobutyric Acid 11

F. Phenylethylamines 12

G. Monoamine Oxidase (MAO) Inhibitors 14

H. N-Alkylated Analogs as Prodrugs 18

H.1 Oxidative N-dealkylation 19

I. Monoamine Oxidase and its Inhibition 22

I.1 Monoamine oxidase - distribution - substrates and inhibitors 22

I.2 Monoamine oxidase and disease states 35

I.3 Monoamine oxidase inhibitors 36

I.3.1 Reversible MAO-A-selective inhibitors 39

I.3.1.1 Harmala alkaloids and tetrahydro-β-carbolines 42

I.3.1.2 Oxazolidinone derivatives 42

I.3.2 Reversible selective MAO-B inhibitors 43

I.3.3 Irreversible inhibitors of MAO 44

I.3.3.1 Cyclopropylamines 48

I.3.4 Mechanisms of inactivation of MAO by (±)-tranylcypromine (TCP) 51

Chapter Page

I.3.5 Metabolism and clearance of TCP 55

I.3.6 Tranylcypromine in psychiatric therapy 58

J. Trace Amines 63

J.1 2-Phenylethylamine (PEA) 63

J.1.1 Metabolic relationship of PEA to other endogenous amines 65

J.1.2 Interactions of PEA with putative neurotransmitter amines 65

J.1.3 Binding sites of PEA 67

J.2 Tryptamine (T) 68

J.2.1 Metabolism of T 70

J.2.2 Interactions of T with putative neurotransmitters 72

J.2.3 Tryptamine receptors 73

K. Analytical Techniques 77

K.1 Gas chromatography (GC) 77

K.1.1 Detectors for GC 78

K.1.1.1 Electron-capture detector (ECD) 78

K.2 Gas chromatography-mass spectrometry 80

K.3 Derivatization for GC-ECD 81

K.4 High pressure liquid chromatography (HPLC) 86

L. Outline of Research Programme, Including Objectives and Hypotheses 94

II. MATERIALS AND METHODS 98

A. Analytical Instrumentation and Apparatus 98

A.1 Gas chromatography 98

A.2 Gas chromatography-mass spectrometry 99

A.3 High pressure liquid chromatography with electrochemical detection (HPLC-EC) 100

Chapter Page

A.4 Liquid scintillation spectrometry 101

A.5 Glassware 101

A.6 Balances 101

A.7 Tissue homogenizer 102

A.8 Centrifuges 102

A.9 Shaker-mixer 102

B. Chemicals 103

B.1 Synthesis of prodrugs of trace amines and their analogs of TCP 107

C. Animals 108

C.1 Administration of drugs 108

D. Analysis of Trace Amines, Prodrugs and MAO Inhibitors 109

D.1 Analysis of PEA and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) in rat brain, blood and liver by extractive pentafluorobenzoylation 109

D.2 Analysis of PEA, N-(3-chloropropyl)-2-phenylethylamine (CPPEA) and N-propargyl-2-phenylethylamine (PGPEA) in rat brain, blood and liver by extractive pentafluorobenzoylation 110

D.3 Analysis of phenelzine (PLZ) in rat brain and patient urine samples by aqueous pentafluorobenzoylation 111

D.4 Analysis of PEA in rat tissues and body fluids by using aqueous pentafluorobenzenesulfonylation 113

D.5 Analysis of TCP and para-fluorotranylcypromine (FTCP) by aqueous pentafluorobenzenesulfonylation 114

D.6 Analysis of T in rat brain, blood and liver samples by aqueous pentafluorobenzenesulfonylation 115

D.7 Simultaneous analysis of PEA, TCP, FTCP and T by aqueous pentafluorobenzenesulfonylation 115

D.8 Simultaneous analysis of T and N-(2-cyanoethyl)-tryptamine (CET) in rat brain, blood and liver samples by aqueous pentafluorobenzenesulfonylation 115

Chapter Page

D.9 Simultaneous analysis of PEA, TCP and N-propargyltranylcypromine (PGTCP) and T by aqueous pentafluorobenzenesulfonylation 116

E. Estimation of MAO Inhibition *In Vitro* and *In Vivo* 116

F. Determination of Rat Brain Levels of Noradrenaline (NA), Dopamine (DA), 3,4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic Acid (HVA), 5-Hydroxytryptamine (5-HT) and 5-Hydroxyindole-3-acetic Acid (5-HIAA) 118

G. Statistical Analyses 119

H. Pharmacokinetic calculations 119

III. RESULTS 121

A. Analysis of Trace Amines, Prodrugs of Trace Amines, TCP TCP Analogs and PLZ 121

A.1 Simultaneous analysis of PEA and CEPEA by extractive pentafluorobenzoylation and GC-ECD 121

A.2 Simultaneous analysis of PEA and CEPEA or PGPEA by extractive pentafluorobenzoylation and GC-ECD 121

A.3 Analysis of PLZ by aqueous pentafluorobenzoylation 130

A.4 Analysis of PEA using aqueous pentafluorobenzenesulfonylation 135

A.5 Analysis of TCP and FTCP by aqueous pentafluorobenzenesulfonylation 135

A.6 Analysis of T by aqueous pentafluorobenzenesulfonylation 135

A.7 Simultaneous analysis of various combinations of trace amines, prodrugs, TCP and TCP analogs 142

A.8 Analysis of catecholamines, DOPAC, HVA, 5-HT and 5-HIAA by HPLC-EC 148

B. Tissue Levels of PEA and its Prodrugs 148

B.1 Tissue levels of PEA after administration of PEA 148

B.2 Tissue levels of PEA and CEPEA after administration of CEPEA 150

B.3 Rat tissue levels of PEA and CEPEA after administration of CPPEA 156

Chapter Page

B.4 Rat tissue levels of PEA and PGPEA after administration of PGPEA 163

B.5 Rat tissue levels of PEA and PGPEA after administration of DPGPEA 164

B.6 Comparison of tissue levels of PEA and its prodrugs 170

C. Inhibition of MAO Following the Administration of Prodrugs of PEA 176

C.1 In vivo and in vitro inhibition of MAO after administration of CEPEA 176

C.2 In vivo and in vitro inhibition of MAO by PGPEA and DPGPEA 176

D. Brain and Liver T Levels Following the Administration of PGPEA 177

E. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA in Rat Brain 177

E.1 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after the administration of PEA 177

E.2 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after administration of CEPEA 183

E.3 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after administration of CPPEA 184

E.4 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after administration of PGPEA 194

E.5 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after administration of DPGPEA 194

F. Tissue Levels of T and CET 195

F.1 Rat brain, blood and liver levels of T following the administration of T 195

F.2 Rat brain, blood and liver levels of T and CET following the administration of CET 208

G. In Vitro and In Vivo MAO Inhibitory Activity of CET 210

H. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA After Administration of T and CET 210

Chapter Page

I. *In Vitro* MAO Inhibitory Activies of Structural Analogs *of TCP* 217

J. Comparative Pharmacokinetic and Neurochemical Studies on TCP and FTCP 225

J.1 Tissue levels of TCP and FTCP after administration of TCP and FTCP 225

J.2 Neurochemical effects of TCP and FTCP 226

K. Pharmacokinetic and Neurochemical Studies on PGTCP 236

K.1 Levels of PGTCP and TCP in rat brain, blood and liver following the administration of PGTCP 246

K.2 Levels of PEA, NA, DA, 5-HT after PGTCP administration 247

L. Analysis of PLZ in Rat Brain and Patient Urines by Extractive Pentafluorobenzoylation 254

IV. DISCUSSION 255

A. Rat Brain, Blood and Liver Levels of PEA and its Prodrugs 255

A.1 Tissue levels of PEA after administration of PEA 255

A.2 Tissue levels of PEA and CEPEA after administration of CEPEA 258

A.3 Tissue levels of PEA and CPPEA after administration of CPPEA 261

A.4 Tissue levels of PEA and PGPEA after administration of PGPEA 263

A.5 Tissue levels of PEA and PGPEA after administration of DPGPEA 267

B. *In Vivo* and *In Vitro* MAO Inhibition Following the *Administration of PGPEA* and DPGPEA 271

C. Levels of T in Brain and Liver Following the Administration of PGPEA 273

D. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA in Rat Brain Following the Administration of PEA and its Prodrugs 273

Chapter Page

E. Discussion of Pharmacokinetic and Neurochemical Effects of PEA and its Prodrugs 257

F. Tissue levels of T and CET 292

G. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA After Administration of T and CET 296

H. Inhibition of MAO *In Vitro* By Structural Analogs of TCP 300

I. Comparative Pharmacokinetic and Neurochemical Studies on TCP and FTCP 303

J. Pharmacokinetic and Neurochemical Studies on PGTCP 319

K. Analysis of PLZ in Rat Brain and Patient Urine Samples by Extractive Pentafluorobenzoylation 324

V. CONCLUSIONS AND RECOMMENDATIONS 327

BIBLIOGRAPHY 333

LIST OF TABLES

Table	Description	Page
1	Parent drug modification and methods of regeneration	4
2	Substrates and inhibitors of MAO *in vitro*	25
3	Relative distribution of MAO activity in rat and human tissues	30
4	Variation in substrate selectivity	32
5	Species and organ differences in substrate selectivities toward MAO	33
6	Changes in platelet MAO activity in psychiatric disorders	37
7	Representative examples of GC-ECD analysis using PFBC and PFBS as derivatizing agents	83
8	Pharmacokinetic parameters of PEA and CEPEA in rat tissues following administration of PEA and CEPEA (0.1 mmol/kg, i.p.)	157
9	Rat brain, blood and liver PEA, CEPEA levels 90 min after CEPEA administration	158
10	Pharmacokinetic parameters of PEA and CPPEA in rat tissues following administration of CPPEA (0.1 mmol/kg, i.p.)	162
11	Pharmacokinetic parameters of PEA and PGPEA in rat tissues following administration of PGPEA (0.1 mmol/kg, i.p.)	168
12	Pharmacokinetic parameters of PEA and PGPEA in rat tissues following administration of DPGPEA (0.1 mmol/kg, i.p.)	173
13	Inhibition of rat brain MAO after administration of CEPEA (0.1 mmol/kg, i.p.)	178
14	*In vivo* inhibition of rat brain and liver following administration of PGPEA, pargyline (PAR), tranylcypromine (TCP) and N-methyl-N-propargyl-2-phenylethylamine (MPGPEA) (0.1 mmol of each/kg, i.p.)	179
15	Inhibition of brain and liver MAO following administration of DPGPEA (0.1 mmol/kg, i.p.)	180
16	Inhibition of rat brain MAO *in vitro* by PGPEA and DPGPEA	181

Table	Description	Page
17	Pharmacokinetic parameters of T and CET in rat tissues following administration of T and CET (0.1 mmol/kg, i.p.)	215
18	Effects of TCP and some of its analogs on inhibition of monoamine oxidase in rat brain *in vitro*	224
19	Pharmacokinetic parameters of TCP and FTCP in rat tissues	231
20	Rat brain and liver *in vivo* inhibition of MAO after injection of FTCP (0.1 mmol/kg, i.p.)	232
21	Rat brain and liver *in vivo* inhibition of MAO after injection of TCP (0.1 mmol/kg, i.p.)	233
22	Levels of PEA in the tissues of control rats and 30 min following the administration of TCP and FTCP (0.1 mmol/kg each, i.p.)	237
23	Pharmacokinetics of TCP and PGTCP in rat tissues after administration of PGTCP (0.1 mmol/kg, i.p.)	250
24	Inhibition of MAO in rat brain and liver after administration of PGTCP (0.1 mmol/kg, i.p.)	251

LIST OF FIGURES

Figure	Description	Page
1	Proposed use of prodrug carrier to transport DA into CNS	10
2	Metabolic pathways of progabide in mouse, hamster, rat, baboon and man	13
3	Structure and mechanism of action of MDL 72394	16
4	Metabolic conversion of 3-amino-2-oxazolidinone to 2-hydroxyethylhydrazine	17
5	Mechanism of N-dealkylation	20
6	Proposed mechanism of inactivation of MAO by TCP	53
7	Structure of midalcipran	54
8	Metabolic pathways of TCP in rat	56
9	Metabolic interrelationships between phenylalkylamines in mammalian brain	66
10	Electrochemical oxidation of DA	90
11	Structures of trace amines and their prodrugs studied	105
12	Structures of TCP analogs studied	106
13	Probable mass spectral fragmentation of the PFB derivative of PEA	122
14	Probable mass spectral fragmentation of the PFB derivative of CEPEA	123
15	A GC trace of an extract of a CEPEA-treated rat brain sample following derivatization with PFBC	124
16	A GC trace of an extract of a CPPEA-treated rat brain sample following derivatization with PFBC	125
17	A GC trace of an extract of a PGPEA-treated rat brain sample following derivatization with PFBC	126
18	Probable mass spectral fragmentation of the PFB derivative of CPPEA	127
19	Probable mass spectral fragmentation of the PFB derivative of PGPEA	129

Figure	Description	Page
20	Typical GC traces of PLZ derivatized with PFBC	131
21	Probable mass spectral fragmentation of the mono-PFB derivative of PLZ	132
22	Probable mass spectral fragmentation of the di-PFB derivative of PLZ	133
23	Typical GC traces of PEA derivatized with PFBSC	136
24	Probable mass spectral fragmentation of the PFBS derivative of PEA	137
25	Typical GC traces of TCP derivatized with PFBSC	138
26	Probable mass spectral fragmentation of the PFBS derivative of TCP	139
27	Probable mass spectral fragmentation of the PFBS derivative of FTCP	140
28	A GC trace of an extract of a T-treated rat brain sample following derivatization with PFBSC	143
29	Probable mass spectral fragmentation of the PFBS derivative of T	144
30	Probable mass spectral fragmentation of the PFBS derivative of CET	146
31	Probable mass spectral fragmentation of the PFBS derivative of PGTCP	147
32	Typical HPLC trace of an extract of brain from a saline-treated rat	149
33	Rat brain PEA levels after administration of PEA and CEPEA	152
34	Rat blood PEA levels after administration of PEA and CEPEA	153
35	Rat liver PEA levels after administration of PEA and CEPEA	154
36	Rat brain, blood and liver levels of CEPEA after administration of CEPEA	155
37	Rat brain, blood and liver levels of PEA after administration of CPPEA	156

Figure	Description	Page
38	Rat brain, blood and liver levels of CPPEA after administration of CPPEA	161
39	Rat brain and blood levels of PEA after administration of PGPEA	165
40	Rat liver levels of PEA after administration of PGPEA	166
41	Rat brain, blood and liver levels of PGPEA after administration of PGPEA	167
42	Rat brain, blood and liver levels of PEA after administration of DPGPEA	171
43	Rat brain, blood and liver levels of PGPEA after administration of DPGPEA	172
44	Availability of PEA in rat brain, blood and liver after administration of PEA and its prodrugs	174
45	Availability of prodrugs of PEA in rat brain, blood and liver after administration	175
46	Rat brain and liver T levels after administration of PGPEA	182
47	Rat brain NA levels after administration of PEA and CEPEA	185
48	Rat brain DA levels after administration of PEA and CEPEA	186
49	Rat brain DOPAC levels after administration of PEA and CEPEA	187
50	Rat brain HVA levels after administration of PEA and CEPEA	188
51	Rat brain 5-HT levels after administration of PEA and CEPEA	189
52	Rat brain 5-HIAA levels after administration of PEA and CEPEA	190
53	Rat brain NA and DA levels after administration of CPPEA	191
54	Rat brain DOPAC and HVA levels after administration of CPPEA	192
55	Rat brain 5-HT and 5-HIAA levels after administration of CPPEA	193
56	Rat brain NA levels after administration of PGPEA	196
57	Rat brain DA levels after administration of PGPEA	197
58	Rat brain DOPAC levels after administration of PGPEA	198
59	Rat brain HVA levels after administration of PGPEA	199

Figure	Description	Page
60	Rat brain 5-HT levels after administration of PGPEA	200
61	Rat brain 5-HIAA levels after administration of PGPEA	201
62	Rat brain NA levels after administration of DPGPEA	202
63	Rat brain DA levels after administration of DPGPEA	203
64	Rat brain DOPAC levels after administration of DPGPEA	204
65	Rat brain HVA levels after administration of DPGPEA	205
66	Rat brain 5-HT levels after administration of DPGPEA	206
67	Rat brain 5-HIAA levels after administration of DPGPEA	207
68	Rat brain T levels after administration of T and CET	211
69	Rat blood T levels after administration of T and CET	212
70	Rat liver T levels after administration of T and CET	213
71	Rat brain, blood and liver CET levels after administration of CET	214
72	Rat brain NA levels after administration of T and CET	218
73	Rat brain DA levels after administration of T and CET	219
74	Rat brain DOPAC levels after administration of T and CET	220
75	Rat brain HVA levels after administration of T and CET	221
76	Rat brain 5-HT levels after administration of T and CET	222
77	Rat brain 5-HIAA levels after administration of T and CET	223
78	Rat brain levels of TCP and FTCP after administration of TCP and FTCP	227
79	Rat blood levels of TCP and FTCP after administration of TCP and FTCP	228
80	Rat liver levels of TCP and FTCP after administration of TCP and FTCP	229
81	Tissue distribution of TCP and FTCP 30 min after administration	238
82	Rat brain levels of PEA after administration of TCP and FTCP	235

Figure	Description	Page
83	Rat blood levels of PEA after administration of TCP and FTCP	239
84	Rat liver levels of PEA after administration of TCP and FTCP	240
85	Rat brain levels of T after administration of TCP and FTCP	241
86	Rat liver levels of T after administration of TCP and FTCP	242
87	Rat brain levels of NA after administration of TCP and FTCP	243
88	Rat brain levels of DA after administration of TCP and FTCP	244
89	Rat brain levels of 5-HT after administration of TCP and FTCP	245
90	Rat brain, blood and liver levels of PGTCP after administration of PGTCP	248
91	Rat brain, blood and liver levels of TCP after administration of PGTCP	249
92	Rat brain, blood and liver levels of PEA after administration of PGTCP	252
93	Rat brain NA, DA and 5-HT levels after administration of PGTCP	253

I. INTRODUCTION

A. Prodrugs

The concept of directing a drug molecule specifically to its target site is an old one, but one which still holds great appeal. This traditionally involved camouflaging the drug in the form of vehicles such as nanoparticles, liposomes, antibodies and synthetic macromolecules. Although impressive results have been obtained *in vitro*, the proper translation of these concepts *in vivo* has been, for the most part, elusive. Drug targeting in its broadest sense means the ability to achieve a desired pharmacological or other response at a selected site without undesired interaction at other sites; this means that either (a) the drug is directed selectively and specifically to the target site where it is concentrated to elicit the desired response or (b) the drug is systemically available but is inactive and activated at only the target site. The vehicular transport approach embraces process (a) whereas enzymatic or chemical reconversion of 'prodrug' or 'precursor' uses process (b). The term 'prodrug' or 'precursor' was coined by Albert (1958) to describe compounds which undergo biotransformation prior to eliciting a pharmacological effect. Harper (1959) used the term 'drug latentiaton' to indicate "the chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound. The chemical alteration of the parent compound is such that the changes in physicochemical properties will affect the absorption, distribution and enzymatic metabolism". The definition was extended by Kupchan *et al.* (1965) to include nonenzymatic or enzymatic conversion to parent drug. The terms 'latentiated drugs', 'bioprecursors', 'bioreversible derivatives', and 'congeners' have been used synonomously with 'prodrugs'. Ideally, the prodrug should be pharmacologically inactive, but this requirement is often difficult to verify in *in vivo* situations, unless the parent drug and prodrug differ markedly in their potencies. Ariens and Simonis (1974) have identified 3 phases in drug action: (1) pharmaceutical phase, (2) pharmacokinetic phase and (3) pharmacodynamic phase; prodrugs have been employed to overcome barriers that minimize the optimum performance of a drug in each of these phases. The barriers include:

(a) economics of drug development
(b) esthetic properties of drug and/or dosage formulation
(c) formulation problems due to physicochemical properties of drug in pharmaceutical phase
(d) bioavailability problems due to either drug or its dosage forms
(e) transport characteristics of the drug
(f) first-pass metabolism
(g) drug toxicity-related local irritation
(h) poor site specificity
(i) too rapid absorption and/or elimination in pharmacokinetic and pharmacodynamic phases.

A number of comprehensive reviews of prodrug literature is available [Harper (1959, 1962), Ariens (1966), Ariens (1971), Albert (1973), Stella (1973), Digens and Swintosky (1975), Higuchi and Stella (1975), Sinkala and Yalkowsky (1975), Sinkula (1975, 1978), Stella *et al.* (1980), Pitman (1981), Poznansky and Juliano (1984) and Gardner and

Alexander (1985)] and no attempt has been made in this thesis to review such exhaustive literature. A brief account of techniques used in the prodrug approach and applications of the approach to psychotherapeutic drugs is given below.

Functional group derivatization has been the cornerstone of the prodrug approach that relies on enzymatic activity in the tissues or on chemical reconversion to reconvert the prodrug to parent drug. Table 1 summarizes the methods of prodrug modification.

The conditions for chemical reconversion of prodrugs (i.e. conversion to parent drug occurs due to presence of water, change in pH, etc.), are somewhat limited and stability during storage could be a problem in addition to lack of tissue-selective and/or -specific targetting. In contrast, prodrugs requiring enzymatic activation could be designed to exploit tissue- or site-specific localization of enzyme, although they are subject to the vicissitudes and vagaries of enzyme induction or inhibition and enzyme-substrate kinetics. The principal factors affecting the success of a prodrug approach would be (1) adequate accessibility of prodrugs to target site, (2) distribution and/or relative activity and specificity of the reconverting enzymes, (3) relative retention of prodrug and parent drug at the target site, (4) pharmacokinetics of prodrug and parent drug, (5) possible pharmacological activity profile of prodrug and/or its other metabolites, and (6) relative importance of alternate metabolic pathways of prodrug. The application of the prodrug approach to different classes of compounds is presented briefly below.

Table 1.—Parent drug modification and methods of regeneration.

		Methods of Regeneration
(A)	Alcohols and Phenols	
(1)	alkyl esters and half esters	E
(2)	phosphate and sulfate esters	E
(3)	sulfoacetyl, dialkylaminoacyl and amino acid esters	E
(4)	acyloxyalkylethers and thioethers	E
(5)	carbamates	E
(B)	Carboxylic Acids	
(1)	alkyl or glycol esters	E
(2)	acyloxyalkyl and lactonyl esters	E
(3)	alkoxycarbonyloxyalkyl esters	E
(4)	(2-oxo-1,3 dioxonolonyl)alkyl esters	E
(5)	amides and amino acid derivatives	E
(C)	Primary, Secondary and Tertiary Amines	
(1)	enamines, Schiff bases, Mannich bases and oxazolidinones	E
(2)	amides and peptides	E
(3)	hydroxymethyl derivatives and their esters	C
(4)	quaternary ammonium salts	E
(5)	carbamates and N-alkyl analogs	E
(D)	Aldehydes and Ketones	
(1)	enol esters	E
(2)	thiazolidines and oxazolidines	C
(E)	Amides and Imides	
(1)	hydroxymethyl derivatives	C
	and their acetate and phosphate esters	E
(2)	Mannich bases	C

E - enzymatic; C - chemically-mediated reconversion (adapted from Gardner and Alaxander, 1985).

B. Neuroleptics

A depot neuroleptic has been defined as one that can be administered in such a form and manner that a single dose affords a therapeutic tissue level for at least a week (Lingjarde, 1973). In many ways the depot neuroleptics are strikingly similar to depot steroids, namely (a) both employ the prodrug derivative as the primary chemical form, (b) the route of administration is intramuscular (as aqueous suspension or drug solution of esters in an oily vehicle), (c) both diffuse slowly from the injection site and experience dilatory metabolism and excretion, and (d) due to their high therapeutic potency, both are ideally suited to depot therapy. Two principal methods of enhancing neuroleptic duration are ester and salt formation. Extremely high oil/water partition coefficients of neuroleptic esters enable them to diffuse slowly from the site of injection into tissue fluids. Esterases in tissues hydrolyse the esters, releasing the parent drug to interact with its site of action, although esterases in blood and brain may also hydrolyse a smaller fraction. Very little prodrug can be detected outside the site of administration. There appears to be a high degree of correlation between the chain length of the fatty acid portion of the ester and the duration of action, with lower aqueous solubility leading to longer duration of effect. The long-acting depot neuroleptics are derived from a variety of chemical classes including phenothiazine, thioxanthene, benzazepine and diphenylbutylpiperidine.

B.1 Phenothiazines

The administration of perphenzine as its enanthate ester resulted in significant urinary levels of perphenazine for up to 4 weeks in patients receiving a 100 mg (i.m.) dose (Van Kempen, 1971). Julou et al. (1973) studied durations of action of undecylenic acid and palmitic acid esters of pipothiazine and the enanthate ester of fluphenazine in dog using a highly sensitive assay (antagonism against apomorphine-induced vomiting). Fluphenazine enanthate exhibited antiapomorphine activity for 5 days, pipothiazine undecylenate for 5-6 days, and pipothiazine palmitate for 13 days; these pharmacological profiles corresponded well with their biological half-lives.

Fluphenazine is a potent tranquilizer chemically derived from phenothiazine that exhibits a short onset (approximately 1 hr) and duration of action (6-8 hr). By chemical modification, duration of action was extended while frequency of administration and the side effects were greatly reduced. Fluphenzine enanthate at a dose of 25-100 mg per month provided adequate therapeutic control in chronic schizophrenics (Schreiber and Grozier, 1973). The decanoate ester of this drug was found to provide satisfactory therapeutic response for up to 4 weeks with significantly reduced extrapyramidal side effects compared to the enanthate ester (Christodoulidis and Frangos, 1975). In a study involving 212 chronic schizophrenics, the use of the decanoate ester of fluphenazine reduced hospital readmissions from 121 to 50 and hospital stays from 8713 to 1335 days (Denham and Adamson, 1971). Similarly, flupenthixol decanoate administered by the i.m. route exhibited a longer duration of action than its parent drug (Nymark et al., 1973).

B.2 Other neuroleptics

Haloperidol is a potent, orally active central nervous system (CNS) depressant, sedative and antipsychotic tranquilizer. It is well absorbed orally and peak plasma levels were observed within 2-6 hr after administration, while elimination was slow with trace quantities of metabolites detectable in urine for up to several weeks. Studies in several species with pimozide (a structurally related analog of haloperidol) indicated that pimozide was much less toxic, with a longer duration of action than haloperidol (Janssen et al., 1968). Another analog, penfluridol, was found to possess a duration of activity approaching almost a week; there was a gradual and smooth onset of action, with peak activity being reached within 24-48 hr. The lack of a 'burst effect' was proposed to reduce toxic side effects seen with many other neuroleptic agents (Janssen et al., 1970).

C. Tricyclic Antidepressants

Imipramine is the original tricyclic antidepressant of the dibenzazepine family useful in treating endogenous and reactive depression (Ball and Kiloh, 1959; Baldessarini, 1985). Wilson et al. (1966) employed the pamoate salt once a day by the oral route in the treatment of depression in 40 patients, with successful results and less side effects than caused by administration of the parent drug itself. Goldberg and Nathan (1972), in a double-blind study in 43 patients with primary depression, observed that a once daily 75 mg dose of prodrug was therapeutically equivalent to three 25 mg doses of parent drug; while this was not dramatic in terms of duration, or even dose, administration of prodrug in this case was considered important in reducing compliance problems.

D. Catecholamines

The adrenergic agent adrenaline (A) has long been employed in the form of eyedrops for the treatment of glaucoma. However, this route of administration was fraught with side effects, both local and systemic, besides problems of poor corneal transport of this hydrophilic catecholamine. The use of dipivalyladrenaline (DPA) resulted in a greater therapeutic effect, and DPA was found to be nearly 100-fold more potent than A in reducing intraocular tension, while producing minimal cardiac effects (McClure, 1975). Dipivalyladrenaline was found to be more lipophilic, with greater penetration across the corneal membrane and with decreased metabolic breakdown compared to A, which principally undergoes conversion to its 3-O-methyl derivative, a reaction catalysed by the enzyme catechol-O-methyltransferase (COMT). Adrenalone (an analog of A in which the secondary alcohol is oxidized to a ketone), a precursor of A, exhibited little activity of its own, but diester (dibutyl, dihexanoyl and diisovaleryl) prodrugs of adrenalone have shown dramatic increases in mydriasis and decreased intraocular tension (Bodor _et al._, 1978). The orally active adrenergic agents terbutaline and N-t-butylarterenol show significant first-pass metabolism. The isobutyl ester of terbutaline (isobuterol) was found to deliver about 60% more terbutaline than did an equimolar dose of terbutaline itself (Hornblad _et al._, 1976), with more reproducible plasma levels. The _para_-methylphenylester derivative of N-t-butylarterenol after oral administration was found to give a prolonged bronchodilator effect (Shargel _et al._, 1976).

In the examples cited earlier, many of the prodrugs, because of their greater lipophilic nature, provided a prolonged pharmacological effect and increased availability at the site of action relative to the drug itself, but this need not always be the case. Dopamine (DA) is not well transported across the blood-brain barrier and, in addition, undergoes significant systemic first-pass metabolism. L-3,4-Dihydroxyphenylalanine (L-DOPA), the precursor of DA, although more polar than DA, is better absorbed because of an active uptake system. The use of L-DOPA in combination with a peripheral aromatic amino acid decarboxylase (AADC) inhibitor in the treatment of Parkinson's disease [caused by degeneration of DA neurons in basal ganglia (Horneykiewicz, 1966)] is one of the best known examples of the therapeutic application of the precursor approach. Because of poor transport characteristics and availability, DA is not a good candidate for replacement therapy. The L-DOPA therapy (combined with peripheral AADC inhibitor) greatly enhances the availability of DA to brain, although this therapy is complicated somewhat by its side effect profile upon chronic administration (Barbeau, 1976). Pinder (1970) suggested the use of O-acylesters such as 3,4-diacetyl- or 3,4-di(trimethylsilyl)dopamines as possible prodrugs of DA in brain. Recently attempts have been made to deliver DA to brain selectively via a carrier. One approach was based on the observation that certain dihydropyridines can pass the blood-brain barrier fairly easily, undergo oxidation to quarternary pyridinium ions and remain trapped in the brain. Bodor *et al.* (1981), Bodor and Farag (1983a), and Bodor and Simpkins (1983) applied this concept to make prodrugs of DA and other centrally active drugs (see Fig. 1 for schematic representation). A similar technique was also applied to 2-pyridine aldoxime methiodide (2-PAM), a drug used to treat poisoning with anti-

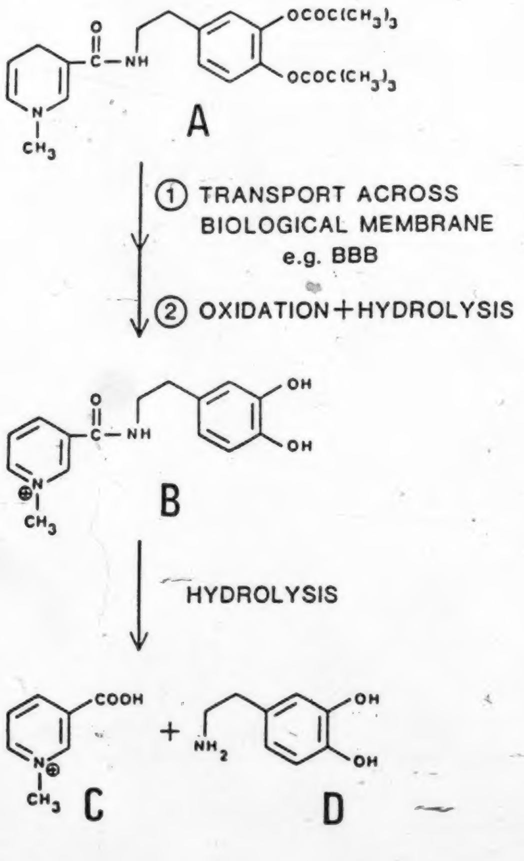

OCOC(CH3)3
OCOC(CH3)3
A
① TRANSPORT ACROSS BIOLOGICAL MEMBRANE e.g. BBB
② OXIDATION+HYDROLYSIS
B
HYDROLYSIS
COOH
C
D

Fig. 1. Proposed use of prodrug carrier to transport dopamine (DA) into the CNS. A: Chemically modified DA; B: DA with carrier trapped in brain; C: oxidized carrier; D: DA (adapted from Gardner and Alaxander, 1985).

cholinesterase nerve gases, to provide enhanced blood-brain barrier penetration (Shek et al., 1976).

Dopamine is known to cause renal vasodilation through activation of specific receptors (Goldberg, 1972), but a relatively high dose has to be employed to achieve sufficient renal vasodilation, and cardiac side effects overshadow the positive pharmacological effect at such doses. Biel et al. (1973) have examined a number of amino acid esters for their breakdown in renal tissues by an aminoacylarylamidase enzyme. N-L-Alanyl- and N-L-phenylalanyl-DA derivatives were too rapidly metabolized to DA in humans, while N-L-isoleucyl,N-L-serinyl esters were very slowly cleaved. Kyncl et al. (1979) observed that it was possible to prepare γ-glutamyl-DA and have it transformed to DA selectively in the kidney because of the presence of higher activity of γ-glutamyltranspeptidase enzyme. Furthermore, nearly complete absorption of γ-glutamyl-DA renders it a good orally active renal vasodilator. An extension of this principle was to employ γ-glutamyl-DOPA which would be broken down to γ-glutamyl-DA by peripheral AADC present in renal tissue; γ-glutamyl-DA can then undergo bioactivation to DA when acted upon by by γ-glutamyl-transpeptidase (Wilk et al., 1978).

E. γ-Aminobutyric Acid

γ-Aminobutyric acid (GABA) is a major inhibitory transmitter present in the mammalian CNS (Meldrum, 1985). The possible involvement of GABA in the mechanisms of action of antidepressants (Pilc and Lloyd, 1984), the etiology of epilepsy and convulsive disorders (Lloyd et al., 1984), Parkinson's disease, Huntington's choreas, Alzheimer's dementia and iatrogenic dyskinetic movement disorders (Fibiger and Lloyd, 1984), and in the anxiolytic actions of benzodiazepines (Martin, 1983; Krogsgaard-Larsen et al., 1984; Meldrum and Braestrup, 1984; Bartholini et al., 1985) has been proposed. Among several agonists of GABA available, a benzylidine compound, progabide (Gabren®) [currently undergoing clinical trials (Bartholini et al., 1985)] was shown to be a potent receptor agonist. A metabolic study of progabide in various animal species, including man (Ferrandes et al., 1985), indicated its breakdown to GABA (thus acting as a prodrug) in addition to its direct agonist action. The metabolic pathway of progabide is shown in Fig. 2.

F. Phenylethylamines

The phenylethylamine nucleus provides the chemical backbone for a variety of biologically active amines, and prodrugs of several phenylethylamines have been examined. Verbiscar and Abood (1970) examined carbamate esters of 2-phenylethylamine (PEA) and various isomers of amphetamine, 4-hydroxyamphetamine and ephedrine. Radiotracer studies in mice with carbamate esters of d-amphetamine revealed delayed onset and prolonged duration of action. Carbamate esters undergo hydrolysis to form carbamic acids which, because of their known instability at physiological pH (Olsen et al., 1952), decompose to generate parent amine and carbon dioxide in a manner analogous to decarboxylation of amino acids to form amines. The alkoxy group in the carbamate ester can act as a carrier, and a variation in the structure of the alkoxy moiety may affect the rate of biotransformation. Because of the low toxicity of

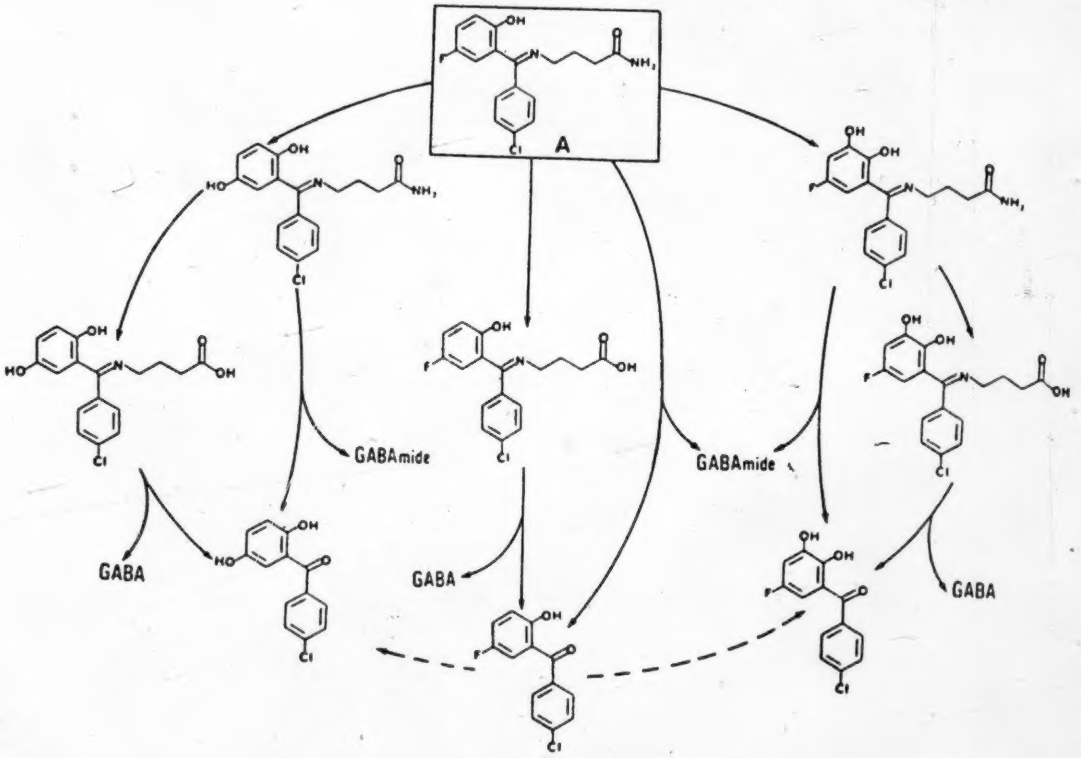

A
GABAmide
GABAmide
GABA
GABA
GABA

Fig. 2. Metabolic pathways of progabide (A) in the mouse, hamster, rat baboon, and man (adapted from Ferrandes et al., 1985).

the ethyl alcohol formed from them, ethylcarbamates were preferred to others. N-Ethylcarbamate analogs of tranylcypromine (TCP), amphetamine and PEA are metabolized in vivo to their parent amines (Baker et al., 1984b).

Several phenylethylamines [amphetamine, PEA, p-tyramine (p-TA)] covalently linked to either starch or polymethylacrylate (Weiner et al., 1972) were examined for their behavioral properties in mice. N-Methylacryloylphenylethylamine monomer was shown to cause sedation for up to 6 hr, whereas adducts to starch caused sedation from 5-24 hr and these durations were significantly longer than those of their monomer counterparts. A dihydropyridine-pyridinium salt type redox system has been used as a brain-specific transport vehicle for PEA in which PEA was coupled to trigonelline (as a quarternary carrier) (Boder and Farag, 1983b; Boder and Abdel Alim, 1985). Following i.v. administration, the PEA-carrier complex continued to accumulate in rat brain for up to 80 min, by which time no complex could be detected in peripheral tissue. In these studies the formation of PEA from the carrier was not measured.

G. Monoamine Oxidase Inhibitors

In the last decade many mechanism-based enzyme inactivators have been developed, and one of the successes has been site-selective enzyme inhibition. Inhibition of both monoamine oxidase (MAO) A and B has been reported to be associated with antidepressant action (Mendlewicz, 1981; Murphy et al., 1981). Inhibition of MAO-A in the periphery was shown to be associated with side effects such as hypotension and the hypertensive crisis seen with concomitant ingestion of TA-containing foods, whereas inhibition of MAO-B did not cause such effects (Finberg et al., 1981; Knoll, 1981). It would be advantageous to design brain-selective MAO inhibitors which would minimize side effects caused by peripheral MAO inhibiton. Several 2-aryl-3-haloallylamine derivatives were shown to be potent enzyme-activated MAO inhibitors in vivo (McDonald et al., 1985). Aromatic hydroxylation at the 4-position gave MAO-A-selective inhibitors, whereas the corresponding 4-methoxy analogs were found to be potent MAO-B-selective inhibitors. The administration of these compounds would not confer any added advantage over the selective A and B inhibitors clorgyline and l-deprenyl respectively as they all produce peripheral MAO inhibition.

Conversion of 2-aryl-3-haloallylamines to their amino acid analogs was found to render them substrates for AADC, resulting in the formation of MAO inhibitors in vivo upon administration of these amino acid analogs. Since it is possible to selectively inhibit peripheral AADC with carbidopa without affecting central AADC, coadministration of amino acid analogs of MAO inhibitors would result in specific conversion to MAO inhibitors in brain (Zreika et al., 1984; Palfreyman et al., 1985). A schematic representation of such a mechanism is shown in Fig. 3.

Another example of a prodrug is 3-amino-2-oxazolidinone (I in Fig. 4) (Fuller and Roush, 1971), a compound without MAO inhibitory activity in vitro but with in vivo inhibitory potency possibly via metabolic conversion to 2-hydroxyethylhydrazine (II) (Fig. 4). 3-Amino-2-oxazolidinone not only leads to more effective inhibition in vivo than does (II), but the selectivity of MAO inhibition in brain versus liver is different for these two compounds. Whereas (II) inhibits liver MAO to a greater extent than brain MAO, (I) causes greater MAO inhibition in

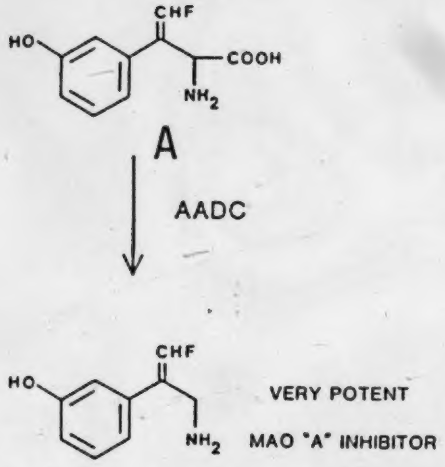

CHF
HO
COOH
NH2
A
AADC
CHF
HO
NH2
VERY POTENT
MAO "A" INHIBITOR

Fig. 3. Structure and mechanism of action of MDL72394 (A) (adapted from Gardner and Alaxander, 1985). Use in combination with a peripheral decarboxylase inhibitor results in central MAO inhibition with minimal peripheral MAO inhibition and also results in minimization of the "cheese effect", i.e. a group of side-effects thought to be caused by accumulation of p-TA in the periphery.

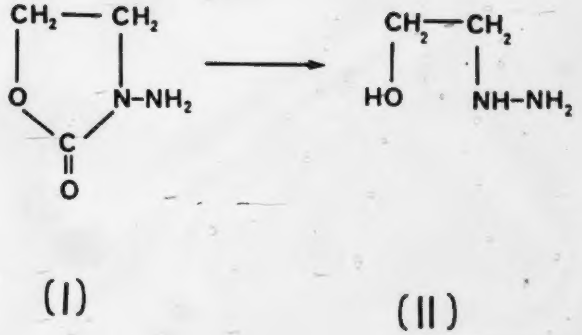

CH₂ — CH₂
O
N-NH₂
C
O
CH₂—CH₂
HO
NH-NH₂
(I)
(II)

Fig. 4. Metabolic conversion of 3-amino-2-oxazolidinone (I) to 2-hydroxyethylhydrazine (II) (from Fuller and Roush, 1971).

brain than in liver. It was speculated that (I) might be acting as a carrier to deliver (II) to the brain.

H. N-Alkylated Analogs as Prodrugs

Substitution of alkyl (or acyl) groups for protons on the nitrogen atom reduces the possibilities of intra- and intermolecular hydrogen bonding, provides an opportunity for introducing a hydrophilic or hydrophobic character to the molecule, and may result in changes in the electronegativity of the nitrogen atom. Consequently, a variety of N-alkylated analogs of amines has been prepared to impart desirable properties.

A methyl substituent is not normally labile in the context of prodrug chemistry, but the following alkyl groups are sufficiently labile under physiological conditions and have been employed in potentially useful prodrugs: hydroxymethyl ($-CH_2OH$), acyloxymethyl ($-CH_2-COOR$), enamine ($-CR^1=CR^2R^3$) and N-Mannich bases ($-CONR_1CH_2NR_2R_3$). N-Hydroxymethylation has been employed to impart greater aqueous solubility and hence dissolution characteristics to phenytoin, uracil, allopurinol, glutethemide and phenobarbital (Pitman, 1981). The product of biotransformation is formaldehyde and, although it is toxic, it is also a product of metabolism of drugs such as methenamine, pivampicillin, rolitetracycline and several N-methyl analogs of drugs. Several acyloxymethyl analogs of pilocarpine have been patented by Boder (1977). N-Mannich bases were proposed as potentially useful prodrugs for anticonvulsant drugs such as hydantoins, barbiturates and succinimides (Pitman, 1981). An enamine analog of the antibiotic cycloserine was found to be a good prodrug _in vivo_ in mice (Jensen _et al._, 1980).

H.1 Oxidative N-Dealkylation

N-Dealkylation of tertiary to generate secondary and primary amines, and of secondary amines to generate primary amines, is one of the most widely observed reactions in metabolism. Even though there is no change in the oxidation state of nitrogen, the removed alkyl group is invariably oxidized. Despite the differences in the alkyl group being removed, one reaction sequence is common to all N-dealkylation reactions of secondary amines: oxidation at an α-carbon generates a carbinolamine intermediate which because of its instability rearranges by N-C bond clevage to yield an N-dealkylated substrate along with a carbonyl derivative of the alkyl substituent. These events are illustrated schematically in Fig. 5.

Although numerous substituents are known to be removed by oxidative N-dealkylation, the following groups have been reported to be metabolically removed in several drugs: methyl ($-CH_3$), ethyl ($-CH_2CH_3$), _n_-propyl ($-CH_2CH_2CH_3$), n-butyl ($-CH_2CH_2CH_2CH_3$), allyl ($-CH_2CH=CH_2$), isopropyl ($-CH(CH_3)_2$), 2-hydroxyethyl ($-CH_2CH_2OH$), 2-cyanoethyl ($-CH_2CH_2CN$) and benzyl ($-CH_2Ph$) (Testa and Jenner, 1976). Oxidative N-dealkylation of amphetamine analogs has been extensively studied in various species (for review see Caldwell, 1976). Significant amounts of amphetamine could be detected in rat tissues and in human urine after administration of N-methyl, N-ethyl, N-n-propyl, N-n-butyl, and N-2-cyanoethyl analogs of amphetamine (Beckett and Shenoy, 1972, 1973; Coutts _et al._, 1972, 1978; Coutts _et al._, 1976a; Coutts and Kovach, 1977; Coutts _et al._, 1986b). Replacement of the N-ethyl group by a 2-cyanoethyl group resulted in marked increases in the extent of N-dealkylation in man (Beckett and Shenoy, 1972). The strong electron-withdrawing capacity of the -CN

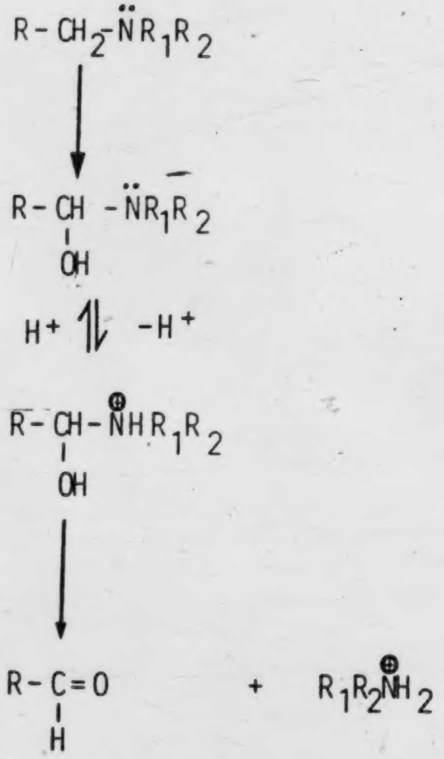


Fig. 5. Mechanism of N-dealkylation.

group dramatically decreases the stability of the N-C bond, which undergoes cleavage under alkaline conditions in vitro (Testa and Jenner, 1976), but the mechanisms responsible for rapid dealkylation in vivo are not clear. Facilitated formation of a carbinolamine intermediate with resultant spontaneous N-dealkylation was reported for 1,4 benzodiazepines containing electron-withdrawing groups at the 1-position [halazepam (CH_2CF_3), flurazepam ($CH_2CH_2NEt_2$) and pinazepam (CH_2-C≡CH)], with desmethyldiazepam being the resultant product (Maksay and Otovos, 1983). These results clearly indicate the influence of electronegative substituents in the alkyl side chain on the rate of N-dealkylation, but generalizations are difficult to make. The 3-chloropropyl group was found to be a rather poor candidate for N-dealkylation despite the electronegativity of the chlorine substituent. A metabolic study of N-3-chloropropylamphetamine (Pondinil®) in different species indicated minor recoveries of amphetamine in rat, rabbit, dog and humans (R.F. Long quoted by Smith and Dring, 1970) after the administration of Pondinil®.

N-Dealkylation generates an additional active hydrogen on the nitrogen and the resulting physical and chemical changes can alter the disposition and biological action of the desalkyl analog compared to the parent compound. For example, primary and secondary amines are more polar than tertiary amines, hence their rates of passive diffusion across lipoidal biological membranes are slower than those of tertiary amines. An active hydrogen on the nitrogen allows formation of N-conjugates that may be eliminated faster than the parent compound (Lindeke and Cho, 1982). The pharmacological consequences of N-dealkylation are difficult to predict. N-Demethylation of the antidepressant imipramine [predominantly an inhibitor of 5-hydroxytryptamine (5-HT)] results in formation of desmethylimipramine (desipramine) which is a predominantly a noradrenaline (NA) uptake inhibitor but is also an effective antidepressant.

During the course of the present investigation, N-2-cyanoethyl, N-3-chloropropyl, N-propargyl and N,N-dipropargyl analogs of PEA, an N-2-cyanoethyl analog of tryptamine (T), and an N-propargyl analog of tranylcypromine (TCP) were examined as possible prodrugs of their respective parent amines.

From the brief discussion presented above it is evident that the prodrug approach has been employed for many classes of drugs either to overcome problems associated with the administration or formulation of parent drug itself, or to achieve site-specific pharmacological effects.

I. Monoamine Oxidase [Amine: Oxygen Oxidoreductase (Deaminating) (Flavin-Containing) EC 1.4.3.4] and its Inhibition

I.1 Monoamine oxidase - distribution, substrates and inhibitors

The presence of an enzyme capable of oxidizing TA in rabbit liver was observed by Hare in 1928 and was given the the name TA oxidase by that researcher. Blaschko _et al._ (1937) studied the deamination of TA, A and PEA in liver, brain and other tissues of mammals, other vertebrates, invertebrates and plants by TA oxidase and suggested the term 'amine oxidase'. Zeller (1951) separated amine oxidases into MAO and diamine oxidase (DAO) depending upon the nature of amines oxidized. The MAO enzymes were found to differ in their prosthetic groups, with the mitochondrial MAO containing a covalently-linked flavin [a derivative of flavine adenine dinucleotide (FAD)] as its only prosthetic group, being devoid of metals or other electron carriers. Plasma amine oxidase, dental-pulp amine oxidase and DAO from placenta were found to contain copper. Because of substrate characteristics, plasma amine oxidase, dental-pulp amine oxidase and aorta amine oxidase have often also been classified as MAO (Kusche, 1983). Monoamine oxidase catalyses the deamination of primary and secondary amines according to the following reaction:

$$RCH_2NHR_1 + O_2 + H_2O \rightarrow RCHO + R_1NH_2 + H_2O_2$$

The substituent R_1 can be either a proton or methyl group. Amines with higher alkyl groups were not found to be favorable substrates for deamination (Blaschko, 1952; Tipton, 1975; Youdim, 1975), but N-oxides of amines were found to be deaminated by MAO (Smith et al., 1962). The attachment of an amine function to a nonsubstituted methylene group appears to be an essential prerequisite, since anilines and α-substituted amines (amphetamines, α-methyltryptamines) were shown to be MAO inhibitors (Mantle et al., 1976b; Blaschko, 1952; Fowler and Ross, 1984). Like many other autooxidizable flavoproteins, MAO reacts directly with molecular oxygen to reduce it to H_2O_2, hence the oxygen atom of the aldehyde carbonyl group originates from water. The first step is the oxidation of the amine to an imine by the protein-bound flavoprotein (FAD) which undergoes reduction in this process. The imine undergoes hydrolysis to an aldehyde and an amine. Molecular oxygen is utilized to regenerate the oxidized FAD. Schematically the reaction can be represented as follows:

$$RCH_2NHR_1 + FAD \longrightarrow RCH{=}NR_1 + FADH_2$$

$$RCH{=}NR_1 + H_2O \longrightarrow RCHO + R_1NH_2$$

$$FADH_2 + O_2 \longrightarrow FAD + H_2O_2$$

In 1968 Johnston observed that clorgyline, an irreversible MAO inhibitor, was able to inhibit oxidation of 5-HT at nanomolar concentrations, whereas the oxidation of benzylamine (BZA) was inhibited at micromolar concentrations. He interpreted this to be due to the existence of two different forms of MAO. Knoll and Magyar (1972) observed an exactly opposite inhibitory pattern for (-)-deprenyl. Based upon the differential inhibition, MAO-A is defined as that form of MAO which is sensitive to clorgyline, whereas MAO-B is that form sensitive to (-)-deprenyl (nanomolar concentrations). On the strength of thermal inactivation experiments, Squires (1972) suggested that the forms were heterogenous. With the identification of two forms of MAO, the endogenous amine transmitters and modulators and inhibitors that were synthesized earlier have been characterized as substrates and inhibitors of either form. Table 2 shows the currently accepted classification of various inhibitors and substrates.

Determination of the molecular weights of purified preparations of the MAO enzyme has given values in the range of 80,000 to 100,000 daltons (Tipton, 1975). At present there is agreement among several groups on a molecular weight of 112,000 ± 2,000, with 2 subunits of 52,000 - 57,000 daltons which are not separable by electrophoresis on polyacrylamide gels. However, only one subunit contains 8α-S-cysteinyl FAD, i.e. there is 1 mole FAD per 112,000 daltons (Singer, 1985).

Many mammalian and avian tissue cells, excluding erythrocytes, contain MAO activity (Blaschko, 1952; Tipton, 1975; and Youdim, 1975).

Table 2. Substrates and inhibitors of MAO *in vitro*.

	MAO-A	Mixed	MAO-B
Substrates	5-HT	NA	PEA, 2-Phenylethanolamine
	A	DA *p*-TA OA (Octopamine) T	o-TA (ortho-Tyramine), BZA, MeHA (Methylhistamine), MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
Irreversible inhibitors	Clorgyline Lilly 51641	Iproniazid Pargyline (PAR) Phenelzine (PLZ) TCP	(-)-Deprenyl Lilly 54781 MDL 72145 AGN 1133 AFN 1135
Reversible inhibitors	Harmaline Cimoxatone Moclobemide CGP-11 305A Amiflamine	Tribulin	MD780236

(from Glover and Sandler, 1986)

Large amounts of MAO activity in liver and intestinal mucosa indicate its possible role in the metabolism of exogenous amines. The enzyme is also present in the blood-brain barrier and is proposed to constitute an important part of it (De La Tore, 1972; Yu, 1984). Monoamine oxidase activity has been shown to be intimately associated with the mitochondrial fraction (Tipton, 1975), although lower activity has also been found in the microsomal fraction; enzymes from these two locations do not differ in their properties (Unzeta et al., 1983). It has been proposed that the microsomal enzyme is derived from cell disruption during fractionation procedures (Jarrot and Iversen, 1968). A significant proportion of MAO activity has also been found to be associated with the endoplasmic reticulum (Muller and Delage, 1977). Because of its faster turnover rate, microsomal MAO has been proposed as the precursor of mitochondrial MAO (Erwin and Simon, 1969), although the observation that MAO synthesis occurs in the cytosol rather than in membrane-bound polysomes appears to contradict the earlier proposal (Sagara and Ito, 1982).

Highest MAO activities in rodents and primates have been found in the liver. Regional variations in brain MAO activity have been observed in many species, with the hypothalamus containing higher activity than the cortex or cerebellum (Blaschko, 1974). In subcellular studies, the crude mitochondrial fraction (consists of myelin fragments, synaptosomes and free mitochondria) contained higher activity than did non-mitochondrial fractions; significant activities were also seen in glial cells, neurons and blood vessels. Reductions in tissue enzymatic activity following lesions with 6-hydroxydopamine or 5,6-dihydroxytryptamine in the periphery (Utresky and Iversen, 1970; Stewart et al., 1979) indicate significant synaptosomal localization. Significant amounts of extraneuronal MAO activity have been demonstrated in liver, kidney, arteries and heart (Boadle and Bloom, 1969; De La Lande, 1970; Horita and Lowe, 1972; Silberstein et al., 1972; Sinha and Rose, 1972; Tipton et al., 1976; Nagatsu et al., 1980).

Species- and tissue-dependent differential distributions of MAO-A and -B enzymes have been examined. Monoamine oxidase activity appears early on in the embryonic life of amphibia, birds and mammals. At birth (or hatch), MAO activity often approaches or exceeds that in adult tissues, although uniform patterns could not be detected in several species. In chick, rabbit and rat, activities decreased during the perinatal period. Several vitamins, minerals and hormones influence the development of MAO (Sourkes, 1979). Iron, once thought a cofactor, is now recognized to be required at some stage of the synthesis of MAO enzyme or its insertion into mitochondrial membrane (Gripois, 1975). In several tissues of bull frog during premetamorphosis, MAO-A activity exceeds MAO-B and during metamorphosis MAO-B increases, indicating that MAO-A activity has an earlier ontogenic development; it was proposed that MAO-B was more essential for terrestial than for aquatic life (Hall and Uruena, 1983). Levinsohn et al. (1980) studied MAO development in humans. Foetal tissues contained relatively low MAO-A activity, but MAO-B activity was of the same magnitude as in adults, with functional maturity of MAO-B being attained at 20 weeks of gestation. In foetal brain, lung, aorta and digestive tract MAO-A appeared much sooner than MAO-B. Adult lung, pancreas, heart and adrenals showed marked increases in MAO-A to -B ratios compared to foetal tissues, whereas brain, spleen and esophagus foetal tissues had higher MAO-A to -B ratios. Among the avian brains examined, new-born pigeon brain was found to contain lowest activity, the chick had about 60% of adult MAO activity, and newly hatched duck and quail had nearly the same enzyme activities as adults (Watts et al., 1964). A number of studies (Baker et al., 1974; Jourdikian et al., 1975; Blatchford et al., 1976; Mantle et al., 1976a; Maderdrut, 1977; Diaz and Samsa et al., 1979; Oreland and Fowler, 1979; Fowler et al., 1980; Strolin Benedetti and Keane, 1980; Leung et al., 1981) examined age-dependent changes in MAO activity in rat, mouse and human brain and they all indicated an age-dependent relative increase in MAO-B activity. In mouse brain, MAO-B activity developed rapidly during the first month of postnatal life, reaching a plateau by 2 months by which time it was nearly 12 times the activity in a newborn; in contrast, MAO-A activity gradually increased in the first month, then decreased slightly (Koide and Kobayashi, 1984). Yu (1984) reported that mitochondrial MAO-A activity increased with age whereas MAO-B activity remained unaltered in rat cerebral blood vessels. Horita (1967, 1968) observed tissue-dependent changes in MAO activity during development even in the same animal. In rat liver, MAO activity during the first week was nearly 20% of adult activity, whereas that in heart was negligible. Females had a slower rate of increase and slightly lower activity than males. In contrast, guinea pig, a precocial animal, showed high MAO activity at birth (Tissari, 1966), with brain and liver activities being 95% of adult values. Even at 25 days of the foetal stage, the activites were nearly 60% of adult levels. Rat brain MAO activity peaked around 15 days after birth and decreased to adult values after weaning. McCaman and Aprison (1964), in a study on rat medulla, observed twice as much MAO activity during the first few days of life as in adult brain, with gradual decreases to adult levels; the authors suggested a caudal-rostral gradient of development of MAO activity. Activity of MAO-B, which usually accounts for 50% of total rat brain MAO activity, was found to be absent in foetal brain 6 days before birth (Mantle et al., 1976a) and absent from some cultured neuroblastoma, glioma and some hepatoma cells (Donnelly et al., 1976; Fowler et al., 1978). A major proportion of total MAO activity was shown to be MAO-A in a number of cell types of neuronal and nonneuronal origin (Hawkins, 1978; Edelstein et al., 1978), and MAO-A activity was found to decrease following sympathetic denervation (Jarrot and Iversen, 1971), indicating localization of MAO-A in the sympathetic nervous system. Table 3 shows the relative distribution of MAO activity in rat and human tissues.

In humans, liver contains the highest specific activity of both forms of MAO followed by kidney, with brain being intermediate. However, if activity is expressed in terms of total tissue weight, skeletal muscle has the highest proportion of MAO-B activity in the body. Human placenta contains exclusively MAO-A while platelets contain primarily MAO-B, and these two tissues have been employed as sources of MAO-A and -B enzymes respectively. The presence of MAO in erythrocytes has not been well established. Blaschko (1952), Tipton (1975) and Youdim (1975) reported an absence of MAO activity in avian and mammalian erythrocytes, but Haddox et al. (1964) suggested small amounts of such MAO activity in children only. In rats, erythrocytes exhibited MAO activity only if a large number of reticulocytes was present (Quiring and Hubertus, 1977). Pig brain, liver and kidney seem to lack MAO-A activity almost totally, whereas pig intensine contains smaller proportions of MAO-A than of MAO-B (Squires, 1972). In general, the MAO-A/-B ratio is high in small intestine of all species studied (rat, rabbit, human, mouse, guinea pig,

Table 3. Relative distribution of MAO activity in rat and human tissues*.

Organ	Rat MAO-A:MAO-B (%)	Human MAO-A:MAO-B (%)
Brain	55:45	40:60
Liver	40:60	55:45
Kidney	70:30	55:45
Heart	>80 (MAO-A)	45:55
Lung	50:50	90:10
Spleen	95:5	35:65
Aorta	<50 (MAO-A)	48:52
Adrenals	>60 (MAO-A)	60:40
Pancreas	>60 (MAO-A)	90:10
Skeletal Muscle	>60 (MAO-A)	30:70
Pineal Gland	15:85	preferentially MAO-B
Intestinal Mucosa	65:35	preferentially MAO-A

(*modified from Tipton et al., 1976 and Glover and Sandler, 1986) cat and dog). In several species (mouse, rabbit, cat, dog, ox and pig), the A form is almost completely absent from liver, while others (rat, guinea pig and human) have considerable liver MAO activity (MAO-A/-B ratio of 0.5). Bovine kidney contains the highest MAO activity (exclusively MAO-B) of all mammals (Gorkin, 1983). Garrick and Murphy (1980) studied brain MAO-A/MAO-B distribution in primates (humans and rhesus and vervet monkeys) and rodents (mouse, rat and hamster). The PEA/5-HT deamination ratio (an index of MAO-B/MAO-A) was 0.8-1.0 in primates, 0.4 in mouse, 0.2 in rat and less than 0.1 in hamster. The species variability in liver MAO activity was much less pronounced, with the PEA/5-HT deamination ratio in vervet, human and rat being 0.26, 0.44 and 0.57 respectively. Platelet MAO activity was also measured in several species; humans and vervet, stumptail and rhesus monkeys (primates) contained exclusively MAO-B activity, rabbit platelets contained 90% MAO-A activity, while no MAO activity (either A or B) could be detected in mouse, rat, cat, hamster, dog, sheep or horse (Garrick _et al._, 1979). Studies in nonmammalian vertebrates (Suzuki and Yagi, 1977; Hall and Uruena, 1983) indicated higher MAO-B in domestic fowl tissues, and almost exclusively MAO-A in many aquatic urodeles and teleosts.

It is widely accepted that PEA and BZA are specific substrates for MAO-B and 5-HT for MAO-A, but such selectivity has not always been maintained. Fowler _et al._ (1981) reviewed the literature between 1968-80 and reported widely varying tissue and species substrate selectivity (see Table 4 and Table 5).

Dopamine metabolism by MAO exhibits marked species differences. Waldmeier _et al._ (1976) investigated the effects of the MAO inhibitors clorgyline and 1-deprenyl on rat striatal DA deamination, and concluded

Table 4. Variation in substrate selectivity of MAO.

Substrate	Number of tissues examined	% of tissues where substrate is oxidized by MAO-A	MAO-A+MAO-B	MAO-B
5-HT	35	61	19	20
p-TA	51	18	57	25
DA	13	29	53	18
Kynuramine	39	12	63	25
PEA	36	6	22	72
BZA	24	0	4	96

*from Fowler *et al.* (1981)

Table 5. Species and organ differences in substrate selectivities toward MAO*.

Tissue	5-HT			*p*-TA			PEA		
	A	A+B	B	A	A+B	B	A	A+B	B
Rat heart	+			+					
Rat brain	+				+				+
Rat liver	+				+				+
Rat vas deferens					+				
Rat skeletal muscle					+				
Rat uterus					+				
Rabbit heart						+	+		
Rabbit lung						+			
Rabbit brain					+				
Cat liver						+			
Dog liver						+			
Chicken heart	+				+			+	
Chicken liver		+			+				+
Chicken liver (adult)								+	
Ox liver			+			+			+
Ox brain					+			+	
Ox heart		+							
Mouse heart					+				
Mouse brain								+	
Moorhen heart			+			+		+	
Moorhen liver			+			+			+
Human heart	+								+
Human brain	+								
Human liver	+								
Human platelet			+			+			+

+: enzyme responsible for deamination

*: from Fowler and Callingham (1978)

that MAO-A was the sole enzyme responsible. Keane et al. (1979) observed DA metabolism by MAO-A in tuberoinfundibular, mesolimbic and nigrostriatal DA neurons. The presence of only MAO-A in nigrostriatal DA neurons was reported by Demerest and Azzaro (1979). Yang and Neff (1974) observed nearly 20% DA deamination by MAO-B in whole brain homogenates, and Garrick and Murphy (1980) clearly demonstrated DA metabolism by B type enzyme in primates (greater than 70%) whereas in rodents the metabolism was preferentially by MAO-A. Primate brains have 70-85% MAO-B compared to 45% in rodents. Recently, guinea pig striatum was shown to contain MAO-A and -B in the ratio of 28:72 and was proposed to be a good model for DA metabolism in humans (Azzaro et al., 1985). Both in man and rat ratios of MAO-A to -B were higher intraneuronally than extraneuronally (Fowler et al., 1984), but there were differences in the extent of DA metabolism. In rat striatum, DA was oxidised extrasynaptosomally 11% by MAO-A and 3% by MAO-B; intrasynaptosomally the values were 84% and 2% by A and B respectively. In humans the corresponding values extrasynaptosomally were 16 and 66% and intrasynaptosomally 12 and 6% respectively (Oreland et al., 1983). This interspecies variation in substrate selectivity should be borne in mind when extrapolating rodent experimental data to clinical situations.

Ever since the discovery of multiple forms of MAO, two different views have been held with regard to the nature of the A and B forms. One school of thought proposes that the two forms are different proteins, i.e. they are separately coded and differ in amino acid sequences, while others hold a view that A and B enzyme activities reflect different active sites on the same enzyme (Mantle et al., 1975; White and Wu, 1975) or the same protein with different lipid attachments

(Houslay and Tipton, 1973). Recently, Cawthorn et al. (1981) provided direct evidence for MAO-A and -B being two distinct isoenzymes by studying ^{3}H-PAR-labelled human platelet, placental and fibroblast MAO using electrophoresis and peptide-mapping techniques. Monoamine oxidase-A (from placenta) and -B (from platelet) were observed to differ in their peptide fragments as well as in their molecular weights (63,000 for MAO-A and 60,000 for MAO-B respectively). Other recent evidence suggests that types A and B MAO are distinct enzyme entities (Brown et al., 1980) and are possibly derived from separate gene loci (Cawthorn and Breakfied, 1979). It would be interesting to know more about mechanisms of tissue- and age-dependent changes in the ratio of MAO-A to -B and to develop cell cultures exhibiting only one type of enzyme activity. Using specific monoclonal antibodies to MAO-A and -B enzymes, Westlund et al. (1985) observed MAO-A to be localized in catecholamine-containing neuronal cell groups, including those in substantia nigra, nucleus locus coeruleus, nucleus subcoeruleus and periventricular regions of hypothalamus, while the MAO-B enzyme was found to be localized in the 5-HT-containing regions nucleus raphe dorsalis and nucleus centralis. Their results indicated similarities between pineal gland and the human platelet which contains a specific 5-HT uptake mechanism along with predominant concentrations of the MAO-B enzyme (Donnelly and Murphy, 1977; Levitt et al., 1982).

I.2 Monoamine Oxidase and Disease States

A number of biogenic amines that control a variety of functions such as behavior, sleep, cognition and memory are metabolized by MAO, and many attempts have been made to correlate disease states with MAO activity. Because of the similarities between brain and human platelet MAO-B (Donnelly and Murphy, 1977), platelets have been used as an index reflecting the neurotransmitter function in the CNS and as a marker in affective disorders. Numerous comprehensive reviews on the role of platelet MAO in disease states are available (Copper et al., 1979; Wyatt et al., 1979, 1980, 1982; Gottfries et al., 1980; Sandler et al., 1981; Fowler et al., 1982a; White et al., 1983; Belmaker, 1984; Maj et al., 1984; Murphy, 1984a; Muscettola et al., 1984; Tachiki et al., 1984; Von Knorring et al., 1984; Siever and Coursey, 1985; Glover and Sandler, 1986) and some of the major findings are enumerated in Table 6.

Mean platelet MAO activity tends to be permanently low in patients with migraine and cluster headache, even when drug-free (Glover et al., 1981) and increased MAO activity has been observed in megaloblastic anemia, Alzheimer's disease and a wide variety of malignant tumors (reviewed by Sandler et al., 1981 and Glover and Sandler, 1986).

In addition to genetic control, MAO activity has been shown to be under hormonal control; activity of the enzyme changes during the menstrual cycle (Sourkes, 1984). Several endogenous modulators of MAO have also been identified in plasma and CSF. These inhibitors appear similar to, if not identical with, tribulin, a compound found in human urine (reviewed in Glover and Sandler, 1986). One has to consider the possible changes in MAO activity due to such normal endogenous factors when analyzing the changes in disease states.

I.3 Monoamine Oxidase Inhibitors

Currently available MAO inhibitors can be divided into several classes based on (1) nature of the inhibition and (2) the type of enzyme

Table 6. Changes in platelet MAO activity in psychiatric disorders.

Findings	References
1. Significantly higher activity in manic-depressives than in schizophrenics	Belmaker et al. (1976)
2. Depressed females have significantly lower activity than depressed males	Friedman et al. (1974)
3. Bipolar depressed have significantly lower activity than controls	Leckman et al. (1977) Landowski et al. (1975) Murphy and Weiss (1972)
4. Bipolar depressed have significantly higher activity than controls	Nies et al. (1974)
5. No difference between bipolar, unipolar and involutive depression (women have higher activity than men)	Takahashi and Karasawa (1975)
6. Lower activity in bipolar depressed than in unipolar	Murphy and Weiss (1972) Landowski et al. (1975)
7. Higher activity in nonendogenous or secondary depression than in primary unipolar depression, depression secondary to borderline personality, bipolar depression and endogenous depression	White et al. (1980) Davidson et al. (1980)
8. Lower activity in cycloid psychotics and in patients with suicidal behavior than in unipolar, bipolar affective disorder patients and unclassified affective psychotics	Gottfries et al. (1980)
9. Low activity correlated with more sensation-seeking	Von Knorring et al. (1984); Murphy et al. (1977)

Table 6. (continued)

Findings	References
10. Low activity correlated with higher use of tobacco and alcohol, with signs of dependence	Von Knorring *et al.* (1984)
11. MAO-B under genetic control	Nies *et al.* (1973) Murphy *et al.* (1976)
12. Lower in schizophrenia and alcoholism than in controls	Major and Murphy (1978) Wyatt *et al.* (1979, 1980, 1982)
13. No difference between control and schizophrenic brain MAO activity (postmortem)	Reveley *et al.* (1981) Meltzer *et al.* (1980)
14. Unipolar depressed have higher activity than controls	Reveley *et al.* (1981)

inhibited. The nature of inhibition can be either reversible or irreversible relating to either MAO-A or -B or both. The classification based on the above features would be:

I. Non-selective irreversible inhibitors
    - iproniazid, TCP, isocarboxazid, PLZ

II. Non-selective reversible inhibitors

III. MAO-A selective irreversible inhibitors
    a. Acetylenic compounds - clorgyline, dinitranyl
    b. Cyclopropylamines - Lilly-51641, Lilly 9393, PCO
    c. Hydrazines - IH-3

IV. MAO-B selective irreversible inhibitors
    a. Acetylenic compounds - PAR, deprenyl, J-508, AGN-1133
    b. Cyclopropylamines - Lilly 54671
    c. Hydrazines - PLZ (human brain)

V. Reversible MAO-A selective inhibitors
    a. α-methyl substituted monoamines - amphetamine, α-methyltryptamine, amiflamine
    b. Harmala alkaloids - harmine, harmaline
    c. Tetrahydro β-carbolines - tetrahydro β-carboline and its analogs
    d. Oxazolidinone derivatives - cimoxatone, toloxatone

VI. Reversible MAO-B selective inhibitors - MD-710222, MD-780236

I.3.1 Reversible MAO-A Selective Inhibitors

Monoamine oxidase deaminates a number of primary and secondary amines that possess an unsubstituted α-methylene group. The initial reaction of MAO has been proposed to abstract a proton from the α-methylene group to generate an imine. Kinetic isotopic studies by Belleau and Moran (1963) revealed that the MAO enzyme abstracts the pro R-proton, and these studies were extended by Yu et al. (1981). Replacement of a proton with a methyl group allows only a two-point interaction instead of a three-point interaction required for catabolism of the amine. Amphetamine has been shown to be an MAO inhibitor (Mantle et al., 1976b) and the S(+)-enantiomer appears to be a more potent MAO-A inhibitor than the R(-)-enantiomer (for discussions on stereospecificity of MAO see Williams, 1982 and Robinson, 1985). Similarly, α-methylbenzylamine has been shown to be a more effective inhibitor of MAO-A than of MAO-B (Mantle et al., 1976b). Tryptamine is considered to be metabolized by both forms of MAO, and α-ethyltryptamine (Monase®) has been demonstrated to be an MAO-A-selective inhibitor (Mantle et al., 1976b) with a proven antidepressant action (Zirkle and Kaiser, 1964).

Recently, 4-dimethylamino-α,2-dimethylphenylethylamine (FLA-336) was reported to be a selective inhibitor of MAO-A in rat (Fowler and Oreland, 1981). This drug is an optically active compound, and the (+)-enantiomer of FLA 336 was shown to be a more potent reversible MAO-A inhibitor than the (-)-form, both in tissues of rats and in human brain samples. It caused selective MAO-A inhibition in rat brain, liver and duodenum, and the enzyme activity recovered within 24 hr (Ask, 1984); this selectivity was maintained even after twice daily administration for up to 2 weeks, and the MAO activity returned to control levels within 24 hr after the last dose (Ask et al., 1984). Using the 5-HT uptake inhibitor citalopram and protection against PLZ-induced inactivation of MAO as an index of MAO inhibition (Green and Elhait, 1980), several

FLA 336 analogs were shown to be selective 5-HT (serotonin) neuronal MAO inhibitors in rat brain (Ask _et al._, 1985). It was proposed that these compounds were transported into serotoninergic neurons by the 5-HT pump. Ask _et al._ (1983) demonstrated that α-ethyltryptamine and _p_-methyoxyamphetamine were also inhibitors of MAO localized in serotoninergic neurons. In the same study, some analogs of FLA 336 were also shown to be inhibitors of MAO in NA-containing neurons, but the inhibition was much weaker than the inhibition in serotoninergic neurons by the same compounds. In both human and rat brain, MAO-A is thought to be localized predominantly, if not exclusively intraneuronally, whereas MAO-B is localized primarily in non-neuronal tissue (Student and Edwards, 1977; Oreland _et al._, 1980; Fowler _et al._, 1980). Neuronal-specific inhibition allows a better understanding of the functional localization of MAO enzymes and may produce greater therapeutic benefits with less side effects. Recent immunohistochemical studies of Westlund _et al._ (1985) indicated a preponderance of MAO-A enzyme in catecholaminergic neurons and MAO-B in serotoninergic neurons in primates. It would be interesting to study the consequences of administration of FLA 336 analogs to primates, although an _in vitro_ study in postmortem human brain samples indicated a selective MAO-A inhibitory potency of these compounds (Fowler and Oreland, 1981).

CGP 11 305A is a benzofuranylpiperidine analog, structurally unrelated to any known MAO inhibitors, and has been demonstrated to be a selective reversible MAO-A inhibitor (Bieck _et al._, 1984). In a recent study in humans, CGP 11 305A at a dose of 75 mg/day was demonstrated to increase excretion of O-methylated metabolites of catecholamines (and to reduce PEA urinary excretion), indicating selective MAO-A inhibition (Maitre _et al._, 1984).

Moclobemide (Ro-1163) is a short-acting inhibitor of the MAO-A isoenzyme through active metabolites formed *in vivo* (Da Prada *et al.*, 1984).

I.3.1.1 Harmala Alkaloids and Tetrahydro-β-Carbolines.

Burger and Nara (1965) observed potent MAO inhibition with the harmala alkaloid harmine. Fuller (1972), in a study of selective MAO inhibition, observed harmaline to be a highly potent MAO-A-selective inhibitor (Ki value of PEA inhibition was nearly 20,000 times higher than that of 5-HT inhibition). Tetrahydro-β-carbolines structurally related to harmala alkaloids have a multitude of pharmacological actions and many of these compounds were demonstrated to be MAO-A selective inhibitors (Meller *et al.*, 1977; Sparks and Buckholz, 1980).

I.3.1.2 Oxazolidinone Derivatives.

Among several oxazolidinone derivatives examined, cimoxatone (MD 780575) and toloxatone were shown to be MAO-A-selective inhibitors (Fowler and Strolin Benedetti, 1983). Dollery *et al.* (1984) observed marked decreases in excretion of deaminated metabolites of NA and A in human volunteers taking 40 mg/day of cimoxatone, with relatively smaller increases in urinary PEA levels than those seen after deprenyl administration, thus indicating MAO-A-specific inhibition.

Cimoxatone at doses of 0.67 - 0.9 mg/kg/day during a 28-day trial showed a highly significant antidepressant activity in 10 depressed patients suffering from a major depressive episode, with significant improvement seen after 2 weeks. A major O-demethylated metabolite (MD770222) of the drug was shown to present in plasma at a concentration

2-3 times higher than that of cimoxatone, and it was proposed to contribute to the overall inhibition of MAO-A observed (Lemperiere *et al.*, 1984).

I.3.2 Reversible Selective MAO-B Inhibitors

In contrast to the availability a large number of reversible MAO-A selective inhibitors, the MAO-B selective inhibitors are few and exhibit only moderate selectivity. Tricyclic antidepressants such as desipramine and nortryptyline inhibited PEA deamination (MAO-B) in human brain in a reversible fashion by about 50% at a concentration of 50 μM (Roth and Gillis, 1974; Roth, 1976). In patients receiving tricyclic antidepressant therapy, significant platelet MAO inhibition has also been observed (Sullivan *et al.*, 1978; Davidson *et al.*, 1981), but the therapeutic significance of this inhibition is not clear. In contrast to the nearly 1000-fold selectivity seen for reversible MAO-A-selective inhibitors (Fowler and Ross, 1984), MAO-B-selective inhibitors such as caroxazone (Moretti *et al.*, 1981) and MD780236 (Tipton *et al.*, 1984a) exhibited only 7-10 fold greater affinity for MAO-B than for MAO-A. Fowler and Ross (1984) suggested these differences might be due to the nature of the interaction of inhibitors with the active sites of enzymes. Monoamine oxidase-A was proposed to contain both an electrophilic and a hydrophobic site, whereas MAO-B has only a hydrophobic site (Severina, 1973; Zeller *et al.*, 1979). Because of these features, MAO-A inhibitors were proposed to experience steric hindrance at the MAO-B active site, hence producing a greater selectivity; an MAO-B inhibitor with a very high degree of hydrophobic site affinity and with a hindered interaction at the MAO-A active site could only achieve moderately selective MAO-B inhibition. This explanation, if proven to be true, suggests that it would be difficult to design MAO-B selective reversible inhibitors, although selective MAO-B-irreversible inhibitors are known.

The drug MD780236 was found to be an MAO inhibitor with selectivity towards MAO-B. *In vitro* it acts as an irreversible inhibitor, whereas *in vivo* the inhibition appears to be reversible. Mechanistic studies revealed that MDL780236 acts both as a substrate for, and as an enzyme-activated irreversible inhibitor of, MAO. It exists as a racemate of (+) and (-) forms (MD240928, MD240931 respectively) and the (+)-enantiomer is a weaker inhibitor of rat liver MAO-B than is the (-)-enantiomer (Tipton *et al.*, 1984a). Interestingly, both enantiomers and the racemate were shown to be substrates for the MAO-A enzyme (Tipton *et al.*, 1983). A comprehensive review of reversible MAO inhibitors has been prepared by Tipton *et al.* (1984b).

I.3.3 Irreversible Inhibitors of MAO

Compounds containing hydrazino, cyclopropylamino and propargyl functionalities have been observed to be selective irreversible inhibitors of both MAO-A and MAO-B by acting as suicidal inhibitors (Singer and Salach, 1981; Tipton and Mantle, 1981). Since the characteristics of inactivation products were proposed to be similar with both forms of MAO (Yu, 1981), the observed selectivities of inhibitors were only explicable in terms of kinetics of interaction with MAO (Green, 1981; Tipton and Mantle, 1981; Fowler *et al.*, 1982b). The nature of the reaction of a suicidal substrate can be represented as follows:

$$E + I \xrightarrow{Ki} EI \xrightarrow{K_2} EI^*$$

where E and I represent free enzyme and inhibitor respectively, EI the noncovalently-bound enzyme inhibitor complex, EI* the covalent enzyme-inhibitor adduct, Ki the kinetic constant for reversible interaction between E and I, and K_2 the kinetic constant for irreversible conversion of EI to EI*.

The kinetics of interaction of PAR, clorgyline and l-deprenyl with MAO were examined in rat liver (Tipton and Mantle, 1981; Fowler *et al.*, 1982b). The Ki value of clorgyline towards MAO-A was nearly 100 times lower than towards MAO-B, and the K_2 value towards MAO-A was nearly 10 times higher than for MAO-B; this means a higher affinity for forming EI with MAO-A than with MAO-B, and faster conversion of EI to El* with MAO-A than with MAO-B. For deprenyl, the Ki value was only 40-fold lower for MAO-B than for MAO-A, but a nearly 7-fold higher K_2 value for MAO-B than for MAO-A confers MAO-B selectivity on deprenyl. For PAR, the Ki value towards MAO-B was only 8 times lower than that for MAO-A and K_2 values for both MAO-A and -B were similar. These observations might explain relatively nonselective MAO inhibition seen *in vivo* after PAR administration, in contrast to deprenyl (Fuller, 1972).

Selectivity of irreversible inhibitors appears to be mainly a function of dose and duration of treatment. At high doses selectivity is lost and both forms of MAO may be inhibited irreversibly; the selectivity of clorgyline has been shown to be less marked *in vivo* than *in vitro*, even though selectivity could be maintained upon chronic administration of low doses (Christmas *et al.*, 1972; Beavan Jones *et al.*, 1972; Yang and Neff, 1974; Campbell *et al.*, 1979; Waldmeier *et al.*, 1981). l-Deprenyl also exhibits similar features (Eksteadt *et al.*, 1979; Prozialek and Vogel, 1978; Waldmeier *et al.*, 1981; Riederer *et* al., 1981). As expected from the kinetic data, PAR exhibits selective inhibition of MAO-B only after acute administration (Fuller, 1972; Della Corte and Tipton, 1980); the studies of Murphy et al. (1978; 1979) and Campbell et al. (1979) indicate the loss of selectivity upon repeated administration.

Selective inhibitors of MAO-B in general (reversible or irreversible) appear to be less selective than MAO-A inhibitors, and Fowler and Ross (1984) have proposed pretreatment with a reversible MAO-A inhibitor such as harmaline to protect the MAO-A active site and thus achieve selective MAO-B inhibition.

During the course of the present investigation, analogs of the cyclopropylamine MAO inhibitor TCP were examined for their MAO inhibition in vitro and in vivo, and N-propargyl analogs of PEA were examined as possible prodrugs of PEA. Swett et al. (1963) examined nearly 100 structural analogs of the N-propargyl MAO inhibitor PAR for their MAO inhibition potencies. Halogen substitution in general increased potency, wheras alkoxy and hydroxy analogs, although active in vitro, were less potent in vivo. Replacement of the benzene ring with heterocyclic rings decreased activity, although the 1-naphthyl analog was highly potent. Compounds in which the methylene group was replaced by ethyl, propyl and isopropyl groups were less active than PAR itself. These workers also prepared deprenyl and reported that its activity was comparable to that of PAR (deprenyl was resynthesized by Knoll et al. in 1965 as 'E-250' and its pharmacological activity examined in detail). Knoll et al. (1978) examined several analogs of l-deprenyl for their MAO-B-selective inhibition; one of them, U-1424 (in which the benzene ring was replaced with a 2-furanyl ring), was observed to be a highly potent MAO-B selective inhibitor. They also concluded that elongation of the chain length between the aromatic ring and the nitrogen atom reduced activity in vitro. Williams and Lawson (1974) examined propargylamines derived from phenylpiperidine and found them to be nonselective MAO inhibitors; in a subsequent study in 1975 they observed a positive correlation between partition coefficient and in vitro MAO inhibitory potency. They also examined N-propargyl-PEA and found it to be a weak MAO inhibitor. Tipton et al. (1982) examined N-propargyl and α-methyl derivatives of amines for their MAO inhibitory activity and concluded that α-methyl substituted primary and secondary amine derivatives tend to be reversible, selective MAO inhibitors. The structural features for selectivity of irreversible inhibitors were more difficult to define as the replacement of the acetylenic group in the MAO-B-selective, irreversible inhibitor J-508 by an ethylenic group rendered it a reversible, MAO-A-selective inhibitor. Swett et al. (1963), however, have observed a loss of activity in a PAR analog in which the acetylenic group was replaced with the ethylenic group. Willams (1984) extended this study by studying α-methyl-substituted analogs of clorgyline and observed retention of MAO-A selectivity in many compounds, thus suggesting that α-methyl group was not uniquely associated with MAO-B-selective inhibitors. Williams (1976) observed that (±)-N-propargylamphetamine, the desmethyl analog of deprenyl, caused MAO-B-selective inhibition in the concentration range of 10^{-4} to 10^{-5} M when rat liver mitochondria were used as a source of MAO. In contrast, deprenyl caused complete inhibition of MAO-B and nearly 45% inhibition of MAO-A at a concentration of 10^{-5} M. This suggests that the N-methyl substituent might not be essential for selective MAO inhibition. Martin et al.

(1975) observed that PAR was nearly 160 times more potent an MAO inhibitor (isoenzyme inhibition was not determined) than N,N-dipropargylbenzylamine (N-methyl group replaced with another propargyl group) in an *in vitro* test, indicating possible steric hindance due to the second propargyl group. Despite these structure-activity relationship studies, no meaningful associations could be made between functional groups and selectivity, and thus it seems likely that the kinetics of interaction determine the selectivity in many instances.

I.3.3.1 Cyclopropylamines. Several cyclopropylamines act as suicidal substrates for MAO but the discussion in this thesis is limited to phenylcyclopropylamines. Tranylcypromine (TCP) is (±)-*trans*-2-phenylcyclopropylamine and can be visualized as a rigid analog of amphetamine. *Trans*-2-phenylcyclopropylamine was shown to be nearly 800 times more potent than the *cis* isomer at inhibiting NA uptake in rat brain hypothalamic synaptosomes and nearly 10 times more potent than the *cis* isomer at inhibiting 5-HT uptake into rabbit platelets (Tuomisto *et al.*, 1973; Tuomisto and Walaszek, 1974). Zirkle *et al.* (1962) undertook a detailed structure-activity relationship study of nearly 60 cyclopropylamines. Inhibition of MAO *in vivo* was examined in terms of T-induced convulsions in rats. The salient features of the study were (1) (+)-TCP was nearly 4 times as potent an MAO inhibitor as was (-)-TCP, (2) the (±)-*cis* isomer was 1/2 to 1/3 as potent as the corresponding (±)- *trans* isomer, (3) increase in the size of the alicyclic ring from cyclopropyl to cyclobutyl, cyclopentyl, or cyclohexyl generally decreased activity in the *trans* series, (4) neither electron-withdrawing nor electron-releasing substituents (Cl, CF_3, OCH_3, CH_3) had much influence on potency, (5) the flat cyclopropyl ring was essential for optimum activity, (6) mono- or disubstitution on the nitrogen with small alkyl groups did not affect the potency, while bulky substituents decreased potency, and (8) N-acylation in general reduced potency, but the benzyl carbamate analog of TCP was found to be more active than TCP itself in vivo [recently the biotransformation of the benzyl carbamate analog to TCP in vivo was demonstrated by Kang and Young, 1984b)].

Finkelstein et al. (1965) have examined the in vitro inhibitory potency of several 2-phenoxycyclopropylamines using TA as a substrate and rat liver mitochondria as the source of MAO. They observed small changes in potency compared to TCP itself, but these changes were not dramatic. Since the discovery of multiple forms of MAO and selective substrates, no studies have been carried out to elucidate structure-activity relationships of cyclopropylamines. During the course of the present investigation such a study was undertaken (see Results section). Therapeutically used TCP exists in racemic form. The separate enantiomers [(+)-TCP and (-)-TCP] as well as (±)-TCP have been examined for their MAO-inhibiting properties. All the three compounds were more effective inhibitors of PEA and TA metabolism than of 5-HT metabolism both in vivo and in vitro (Fuller, 1968; Fuentes et al., 1976). The order of ranking of MAO-inhibitory potency was (+)-TCP>(±)-TCP>(-)-TCP (Zirkle et al., 1962). This original observation has been confirmed both in vivo (rat and human brain) and in vitro (Fuentes et al., 1976; Reynolds et al., 1980; Hampson et al., 1986; and present investigation), the difference between the isomers was much less marked in vivo than in vitro (Reynolds, 1981) possibly because of pharmacokinetic differences between the two enantiomers. Platelet MAO inhibition data in healthy individuals and Parkinson patients (single as well as multiple dosing of TCP isomers) indicated greater MAO inhibition after (+)-TCP than after (-)-TCP (Reynolds et al., 1980; Reynolds et al., 1981). In a study attempting to relate antidepressant activity of MAO inhibitors to their ability to inhibit uptake of neurotransmitter amines, (-)-TCP was found to be a more potent catecholamine uptake inhibitor (Horn and Snyder, 1972) than was (+)-TCP; similar results were obtained by Hampson et al. (1986). As DA uptake inhibitors, both isomers and (±)-TCP were of similar potency (Hendley and Snyder, 1972). Recently, Tuomisto and Smith (1986) reported that (+)-TCP was more potent than (-)-TCP at inhibiting 5-HT uptake in rat brain synaptosomes, whereas (-)-TCP was more potent than (+)-TCP at inhibiting NA and DA uptake. Hampson et al. (1986) observed that (-)-TCP was more potent than (+)-TCP at inhibiting uptake of NA, DA and 5-HT; the enantiomers differed only slightly in their potency to release these monoamines. Consistent with their finding of its potency at inhibiting 5-HT uptake, Tuomisto and Smith (1986) reported that (+)-TCP was more potent at inhibiting [^{3}H]-imipramine binding to rabbit platelets than was (-)-TCP. Configurational analysis indicated that (+)-TCP has a (1S, 2R) configuration which is similar to that of the phenylethylamine moeity of the 5-HT agonist (+)-LSD, while the (-)-TCP has a (IR, 2S) configuration similar to the phenylethylamine moiety of the DA agonists (-)-apomorphine and (-)-5,6-dihydroxy-2-aminotetralin. The configurations were proposed to favor (+)-TCP's serotoninergic mechanisms, and (-)-TCP's catecholaminergic mechanisms; such findings were supported by the behavioural effects of the enantiomers (Smith, 1980) and neurochemical effects such as actions on uptake and release of NA, DA and 5-HT and effects on the imipramine binding site (Tuomisto, 1978; Smith and Petersen, 1982; Tuomisto and Smith, 1986).

Lang *et al.* (1979) reported that, in humans receiving 20 mg of either (+)- or (-)-TCP, (-)-TCP attained higher peak plasma levels and was eliminated at a slower rate than (+)-TCP. Fuentes *et al.* (1976) also observed higher peak levels in rat brain of (-)-TCP than of (+)-TCP following administration of the separate isomers. Hampson *et al.* (1986), in a more prolonged time study, observed higher peak levels for (-)-TCP in rat brain, but (+)-TCP appeared to be eliminated at a slower rate than (-)-TCP. Hendley and Snyder (1968) proposed that the antidepressant action of TCP may involve inhibition of amine uptake rather than MAO inhibition, which supports the reported increased therapeutic efficacy of (-)-TCP over (+)-TCP (Escobar *et al.*, 1974); however, this idea remains controversial [Gentil *et al.* (1978)]. Both MAO inhibition and amine uptake inhibition may contribute to the overall therapeutic profile of TCP (Maxwell and White, 1978) and further studies are required to elucidate the mechanisms.

I.3.4. Mechanisms of Inactivation of MAO by TCP

Paech *et al.* (1980) have proposed that TCP is a mechanism-based MAO inactivator. They suggested that the initial reaction step was the oxidation of TCP to an imine and that this imine, or the ketone product of hydrolysis, is attacked by the thiol group at the site of the enzyme to form a thiohemiketal or a thioaminoketal stabilized by the tertiary structure of the native enzyme. In an *in vitro* study, the nature of the oxidized inhibitor was determined to be 2-phenylcyclopropanone. The mechanism is represented in Fig. 6. Because of the known instability of cyclopropanones, Silverman (1983) reexamined the mechanisms of MAO inactivation and the nature of oxidized substrate. Using 2,4-dinitrophenylhydrazine to derivatize the oxidized substrate under acidic conditions, the oxidized substrate was determined to be cinnamaldehyde, not 2-phenylcyclopropanone as previously proposed. The mechanism is represented in Fig. 6. Similar reaction pathways have also been proposed for 1-benzylcyclopropylamine and 1-(phenylcyclopropyl)methylamine (Silverman and Zieske, 1985). The mechanism would essentially require attachment of the amino group onto the cyclopropyl moiety; a corollary would be that the compounds wherein the cyclopropyl moiety is separated from the amino group by a methylene group would not support the mechanism, and hence they may not act as a suicidal inhibitors.

Recently, midalcipran, a fourth-generation antidepressant drug currently undergoing clinical trials, has been demonstrated to be lacking any MAO inhibitory activity [_in vitro_ and _in vivo_ (at a dose of 100 mg/kg, i.p.)]. Midalcipran (Fig. 7) is a phenylcyclopropylamine antidepressant which is a potent inhibitor of NA and 5-HT uptake without affecting DA, without affinity for α or β-adrenergic, muscuranic, histaminergic H_1, dopaminergic D_2 or serotoninergic 5-HT_2 receptors and without effect on β-adrenoceptors even after chronic administration (Moret _et al._, 1985). It seems likely that the bulky diethylaminocarbonyl substituent at the 1-position sterically hinders interaction with the MAO active site. Further studies are required to determine structural requirements for effective antidepressant action in this group of compounds.

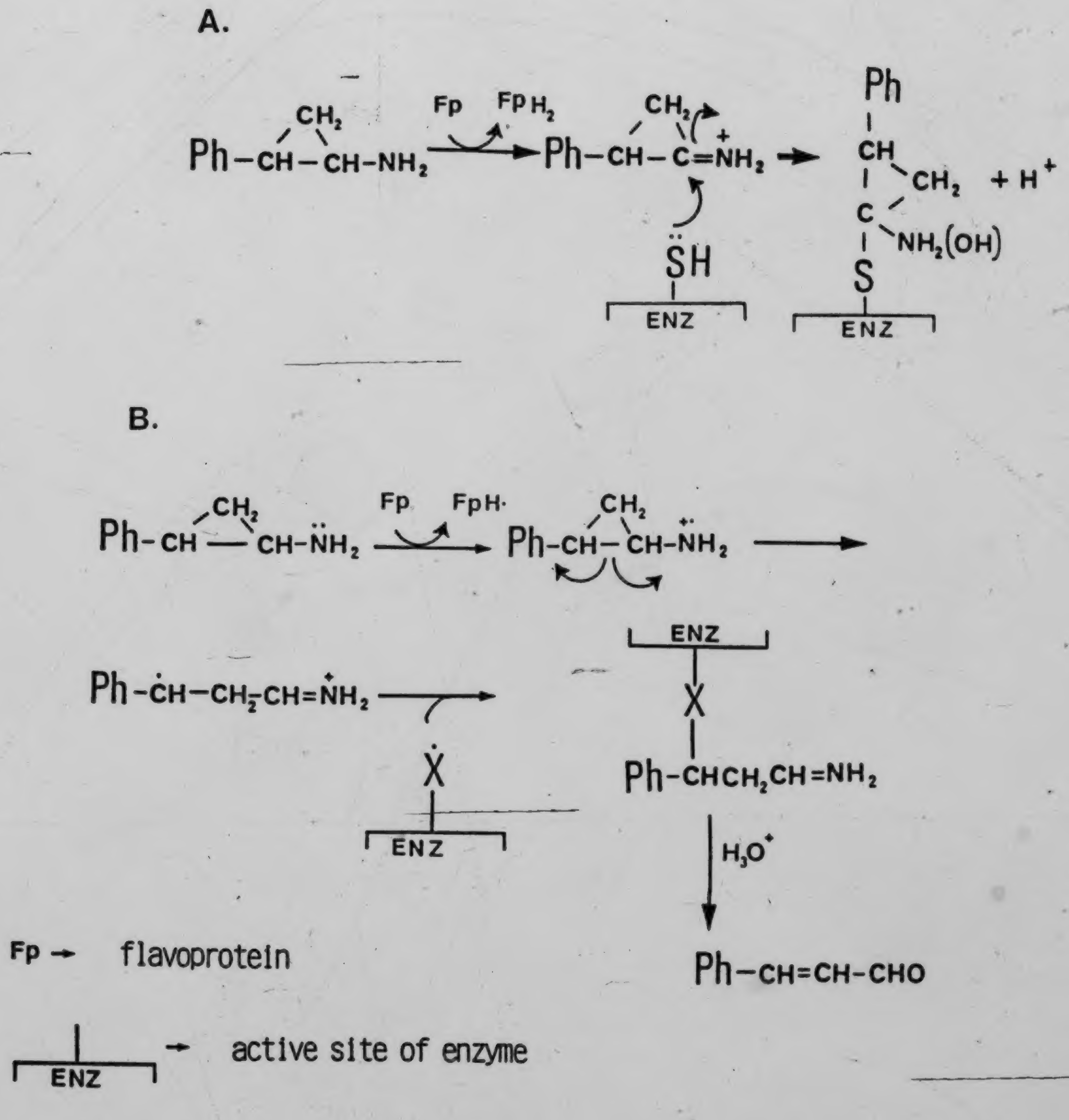

A.
Fp
FpH₂
Ph
CH
CH₂
+ H⁺
C
NH₂(OH)
SH
S
ENZ
B.
Fp
FpH·
H₃O⁺
Ph-CH=CH-CHO
X
Fp → flavoprotein
→ active site of enzyme

Fig. 6. Proposed mechanisms of inactivation of MAO by TCP.
A: Mechanism proposed by Paech et al. (1981)
B: Mechanism proposed by Silverman (1983)
Ph: phenyl ring

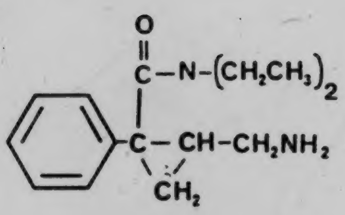


Fig. 7. Structure of midalcipran

I.3.5 Metabolism and Clearance of TCP

Despite the use of TCP in therapy for the last two decades, very few metabolic studies have been conducted on this drug. Alleva (1963) reported that administration of ^{14}C-labelled TCP to rats resulted in excretion of about 4% TCP unchanged and 12% as hippuric acid; 4 further ^{14}C-labelled metabolites were present but were not characterized. Calverley *et al.* (1981) have identified N-acetyl-TCP as a metabolite in rat brain.

Studies of amphetamine metabolism revealed that aromatic hydroxylations occurred, resulting in formation of phenols and of catechols which were metabolized further by O-methylation to give 3-methoxy-4-hydroxyamphetamine (Coutts *et al.*, 1976b). Other important pathways were hydroxylation on the aliphatic side chain, and deamination (for a review of amphetamine metabolism see Caldwell, 1976). Because of the structural similarity to amphetamine, TCP would be expected to undergo aromatic hydroxylation. Baker *et al.* (1986b) detected *para*-hydroxytranylcypromine (HTCP) in rat urine and brain. Kang and Young (1984a) identified N-acetyl and hydroxylated N-acetyl-TCP and Kang and Choi (1986) reported identification of the glucuronide conjugate of hydroxylated N-acetyl-TCP in rat urine samples. The metabolic pathways of TCP in rat are represented in Fig. 8.

Baker *et al.* (1986b) characterized the MAO inhibitory properties of HTCP. These studies indicated HTCP to be weaker MAO inhibitor than TCP itself, but with more selectivity towards MAO-A than to MAO-B. Kang and Choi (1986) administered rats N-acetyl-TCP (0.4 mmole/kg, i.p.) and observed a gradual increase in MAO inhibition in brain (about 90% by 6 hr and 74% after 24 hr). This was in contrast to nearly 94% MAO inhibi-

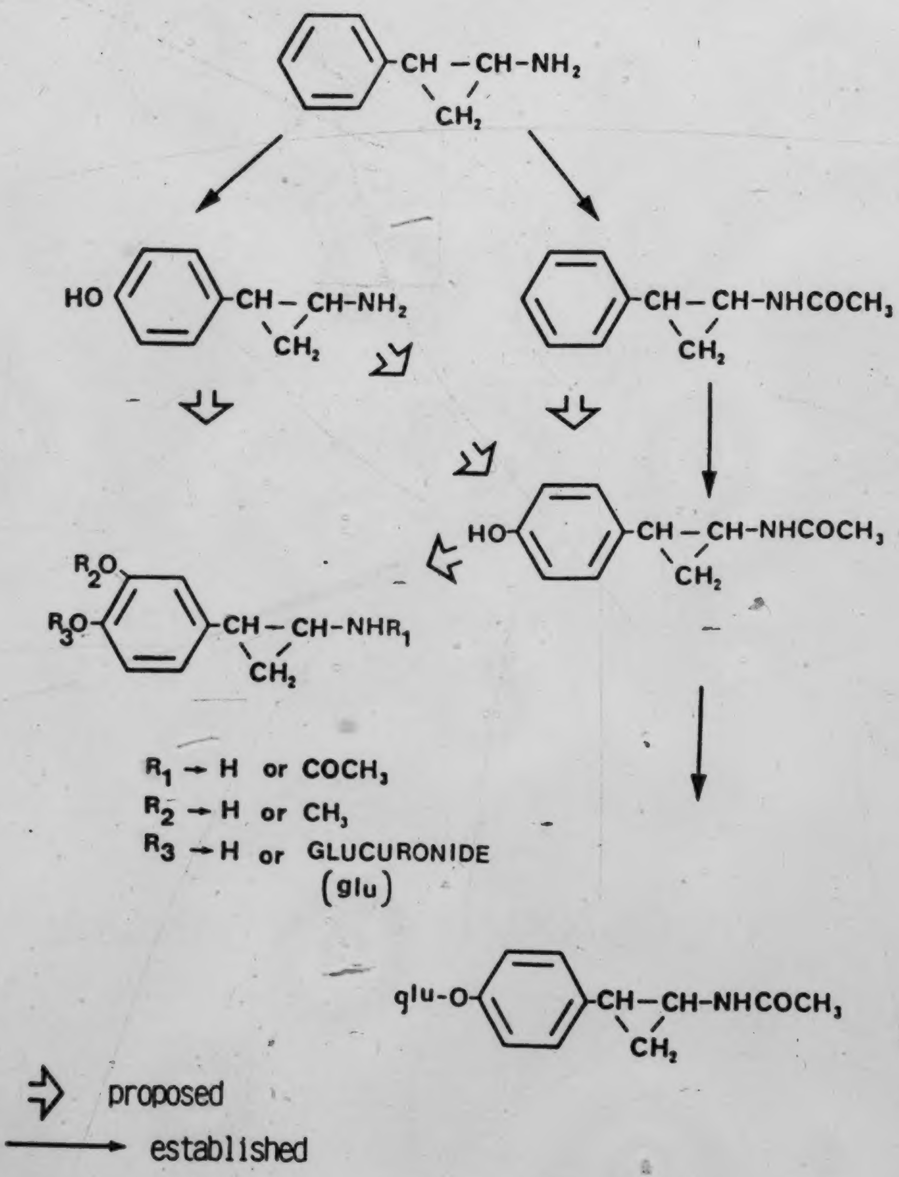

CH – CH-NH₂
CH₂
HO
CH – CH-NH₂
CH₂
CH – CH-NHCOCH₃
CH₂
HO
CH – CH-NHCOCH₃
CH₂
R₂O
R₃O
CH – CH-NHR₁
CH₂
R₁ → H or COCH₃
R₂ → H or CH₃
R₃ → H or GLUCURONIDE (glu)
glu-O
CH–CH-NHCOCH₃
CH₂
proposed
established

Fig. 8. Metabolic pathways of TCP in the rat.

tion within 0.5 hr of administration of TCP (0.015 mmol/kg, i.p.), which decreased to 49% by 24 hr. The authors considered this as indirect evidence of *in vivo* bioactivation of N-acetyl-TCP to TCP. Based on the area under the *in vivo* MAO inhibition-versus-time curves after administration of TCP and N-acetyl-TCP , the authors proposed that 3-4% of administered N-acetyl-TCP was converted to TCP. This assumption could be criticized on the grounds that for irreversible enzyme inactivators such as TCP, after an initial inactivation step, inactivation is not proportional to concentration of the inhibitor. Only an *in vivo* study in which measurements were made of levels of both N-acetyl-TCP and TCP formed from it would reveal the exact extent of biotransformation. The authors also speculated that polymorphic N-acetylation of TCP might be responsible for the lack of clinical response in some patients (because of excessively rapid elimination) compared to others. It is also likely that some of these metabolites might affect uptake and release of monoamine neurotransmitters and that these effects may contribute to the overall therapeutic profile of TCP.

Youdim *et al.* (1979) reported the presence of amphetamine in a patient who had taken an overdose of TCP, but subsequent studies (Reynolds *et al.*, 1980; Hampson, 1984) could not substantiate the earlier claim.

Tranylcypromine is structurally similar to amphetamine and PEA, and like those two compounds, reaches very high concentrations in brain following i.p. injection and then disappears from that organ rapidly. One of the objectives of the project described in this thesis was to examine analogs of TCP that might give rise to more sustained, consistent levels of TCP in brain.

I.3.6 Tranylcypromine in Psychiatric Therapy

Tranylcypromine's nonhydrazine structure and structural similarity to amphetamine differentiate it from other MAO inhibitors such as phenelzine, isocarboxazid and iproniazid which have also been used clinically. Early clinical trials with TCP indicated clinical efficacy (Morris and Beck, 1974), with rapid onset of psychotherapeutic effect being reported in some patients (Lesse, 1978). Many of the clinical studies that were conducted with TCP involved groups of depressed patients with diagnostic heterogeneity or chronic schizophrenics in which therapy involving a combination of TCP with the antipsychotic tri~~flu~~fluperazine (a combination marketed as "Parstelin") was used; the evaluation methodologies often fell short of current standards (White et al., 1984). Atkinson and Ditman (1965) reviewed the TCP literature, including 54 controlled and uncontrolled clinical trials. Among these, in only 16 trials was TCP alone administered to a group of primarily depressed patients. The reviewers concluded that the evidence indicated that TCP was efficacious in psychoneurotic or reactive depressions, but of doubtful value in endogenous, involutional or agitated depressions. Various studies estimated the incidence of hypertensive syndromes from 2-25%, although a worldwide compilation revealing only 500 reported cases among a population of 3.5 million patients suggests a much lower incidence (0.02%) (White et al., 1984). Gottfries (1963) treated 50 depressed inpatients for 2 weeks with either TCP or placebo, with only 24% of TCP-treated and 8% of placebo-treated patients showing improvement. Richmond and Roberts (1964) randomly assigned 80 depressed outpatients to a open 3-week trial of either TCP, isocarboxazid, imipramine or amitriptyline, with failure to respond to one treatment resulting in a switch-over to another drug (thus resulting in a total of 146 different drug trials). Among the patients, 20 were reactive depressives who responded rather infrequently to all treatments (20% improvement); the authors concluded that the TCP and isocarboxazid groups combined had significantly greater improvement (70%) than did imipramine and amitriptyline groups combined (33%). Himmelhoch _et_ _al._ (1982) indicated significant advantages to the use of TCP in anergic depression. Razani _et_ _al._ (1983) reported therapeutic equivalence of TCP and amitriptyline in treating depressed patients. White _et_ _al._ (1984) reported that TCP offered an effective and reasonably safe treatment for nonendogenous depression, although a significant advantage over tricyclics in treating this disorder has yet to be demonstrated. Other studies indicated the usefulness of TCP in treating depressions associated with anxiety, agitation and phobias (Lesse, 1978; Sheehan _et_ _al._, 1980; Tyrer, 1976; Jenike, 1984). A combination of TCP with desipramine has been reported effective in treating depression associated with anxiety (Raskin, 1983). Jenike (1981) and Jenike _et_ _al._ (1983) found that TCP produced a rapid response in obsessive-compulsive disorder associated with anxiety, and Walsh _et_ _al._ (1982) found TCP useful in treatment of bulimia. Price _et_ _al._ (1985a) found a combination of TCP and lithium effective in treating major depression refractory to other (non MAO inhibitor) antidepressant treatment, and their preclinical studies of a combination of lithium and an MAO inhibitor indicated a activation of serotoninergic pathways that may play a role in therapeutic efficacy. They extended their studies (1985b) to demonstrate significant increases in prolactin response to tryptophan in the presence of TCP, thus suggesting enhancement of serotoninergic function by TCP. Behavioral effects of TCP

(Smith, 1980) and the beneficial effects of TCP in obsessive-compulsive disorder and bulimia point out the possible importance of serotoninergic mechanisms in the pharmacological action of TCP (further discussion is presented in chapter IV).

Since the resurgence of interest in MAO inhibitors, numerous reviews have been published covering various aspects of these drugs (Finberg and Youdim, 1983; Sandler, 1983; Tollefson, 1983; Zemishlany *et al.*, 1983; Fowler and Ross, 1984; Mann *et al.*, 1984; Murphy *et al.*, 1984b; Murphy *et al.*, 1984c; Goodman and Charney, 1985; Pare, 1985; Zisook, 1985; McDaniel, 1986; Youdim and Finberg, 1986). Nies (1983) described the typical symptom profile of patients responsive to MAO inhibitor therapy:

1. Psychopathological symptoms: mood reactivity retained, irritability, panic episodes, agoraphobia, social fears, hypochondriasis, obsessive preoccupation
2. Interpersonal reactions: self pity/blaming others, communicative suicidal actions, rejection sensitive, vanity/applause seeking, histrionic personality
3. Vegetative symptoms: initial insomnia, hypersomnia, weight gain, hyperphagia, craving for sweets, lethargy and fatigue, tremulousness
4. Historical features: personal loss before intensification, poor response to electroconvulsive shock therapy, liking amphetamines, dysphoric tricyclic responses, alcohol/sedative abuse.

For several years, therapeutic combinations of an MAO inhibitor with a tricyclic was considered highly dangerous, but reviews of such treatment [Schukit *et al.* (1971), Sethna (1974), Ananth and Luchins

(1977) and White and Simpson (1981)] suggest that, provided drugs are given properly with regard to dose and sequence, the incidence of adverse effects other than weight gain and orthostatic hypotension is no higher than with either drug treatment alone.

Monoamine oxidase inhibitors have been employed in the therapeutic management of various conditions that include reactive, neurotic or atypical depressions; depressive anxiety states; atypical facial pain; phobic anxiety, e.g., agoraphobia; the phobic depersonalization syndrome; refractory thought disorders; neurodermatitis; treatment-resistant narcoleptic states; recalcitrant migrainous headache (associated with reduced plasma 5-HT) and idiopathic orthostatic hypotension (Tollefson et al., 1983). These drugs may be adjunctive to an antihypertensive protocol (Gibbs, 1966), although the mechanism is not clear.

The development of isoenzyme-specific inhibitors raised new hopes of improving the antidepressant action of MAO inhibitors while decreasing the side effects of irreversible inhibitors. In early clinical trials, the antidepressant activity of clorgyline was demonstrated (Murphy et al., 1981). However, clorgyline does not offer any unique advantage over nonspecific MAO inhibitors because there are still considerable side effects. For MAO-B inhibitors, the situation is less clear. Even though PAR (Murphy et al., 1981) and caroxazone (Conti et al., 1980) have been demonstrated to possess antidepressant action, both MAO-A and -B were inhibited at the doses employed. In one double-blind study, (-)-deprenyl was ineffective in treating a group of endogenous depressives (Mendis et al., 1981). However, this drug has been reported to be effective in nonendogenous depression (Mann et al., 1982).

Mendlewicz et al. (1981) demonstrated the usefulness of (-)-deprenyl in combination with 5-hydroxytryptophan. In this study, the authors employed a dose of (-)-deprenyl of 5 mg/day. In Parkinsonian patients receiving 10 mg/day of deprenyl, autopsied brain samples indicated significant inhibition of 5-HT deamination (ranging from 38% in putamen to 84% in hypothalamus) (Riederer et al., 1981), so it would be difficult to ascribe the observed therapeutic benefit solely to MAO-B inhibition. In a recent review of clinical trials with selective irreversible and reversible MAO inhibitors, Mann et al. (1984) concluded that (-)-deprenyl may be most effective for patients with nonendogenous depression and vegetative signs, but not particularly useful for patients with associated panic attacks and phobic symptoms. A combination of (-)-deprenyl (5-10 mg/day) and phenylalanine (250 mg/day) has been shown to be useful in unipolar depression (Birkmayer et al., 1984).

The combination of L-DOPA with a peripheral AADC offered great promise in the treatment of Parkinsonism, but many patients (30-40%) receiving this therapy chronically show "on-off" phenomena (periodic reemergence of akinesia followed by periods of improved mobility) (Youdim et al., 1984). It is possible that this apparent lack of therapeutic effect could be due to either (1) decreased uptake of L-DOPA into dopaminergic neurons and/or (2) rapid metabolism of DA by MAO; in this latter case it would be desirable to add an MAO inhibitor to prolong the activity of DA formed from L-DOPA. Addition of (-)-deprenyl to L-DOPA along with a peripheral AADC resulted in marked improvement in Parkinsonian patients in the "on-off" period (Birkmayer et al., 1975), and a number of clinical studies reported thereafter confirmed this observation (reviewed in Youdim et al., 1984).

Despite their chequered history, MAO inhibitors form an established part of the therapeutic armamentarium of drugs used in treating psychiatric disorders. With the development of isoenzyme-selective and neuronal-selective MAO inhibitors it is possible to study more comprehensively the role of MAO in health and disease, enabling us to better understand the nature of psychiatric illnesses.

J. Trace Amines

J.1 2-Phenylethylamine.

Classical neurotransmitters such as NA, DA, 5-HT and acetylcholine (ACh) have been implicated in the pathophysiology of affective disorders and schizophrenia (Bunney and Garland, 1981; Enna and Kendall, 1981; Snyder, 1981; see Baker and Dewhurst, 1985 for a review). For a long time the functional significance of endogenous amines such as PEA, T, TA and OA which occur in very low absolute concentrations in the central nervous system [hence named "trace amines" (Usdin and Sandler, 1976)] had been neglected. Based on a large body of experimental evidence, Dewhurst (1968) proposed functional deficiences of PEA and/or T as possible etiological factors in depression; he also underlined the importance of receptor changes and psychosocial stimuli. Several groups subsequently proposed a deficiency of PEA in depression (Boulton and Milward, 1971; Fischer *et al.*, 1972; Sabelli and Mosnaim, 1974; Sandler *et al.*, 1979). Low absolute concentrations coupled with methodological problems associated with unequivocal quantitation of these trace amines had led to their relative neglect. Developments in analytical methodology over the past 15 years resulted in renewed interest in their role in brain function. A survey of the literature indicates that several research groups found that urinary PEA levels in depressives were significantly below control levels (Boulton and Milward, 1971; Fischer and Heller, 1972; Sabelli and Mosnaim, 1974), but this still remains somewhat controversial, probably largely due to the somewhat heterogenous groups of depressed patients investigated in some of these studies. Wyatt _et al._ (1977) and Lauber and Waldmeier (1984) reviewed urinary PEA excretion studies which had been conducted in healthy controls and depressed patients. In a study involving 15 patients undergoing MAO inhibitor treatment, Murphy _et al._ (1984d) concluded that pretreatment urinary excretion of PEA was not consistently associated with differences in severity of depression, anxiety activation or hypomania, but was negatively correlated with dysphoria and anger; during treatment increased urinary excretion was correlated with decreased self-rated depression and anxiety. These workers suggest that their data might be consistent with an amended hypothesis that relates PEA to a cluster of depression-related symptoms (such as dysphoria and anger), but not to depressed mood alone.

Administration of MAO-inhibiting antidepressants causes marked increases in brain levels of trace amines, in particular PEA and T, the increases being many times greater than those for putative neurotransmitters such as DA, NA and 5-HT (Boulton _et al._, 1973; Axelrod and Saavedra, 1974; Boulton, 1976a; Philips and Boulton, 1979; Baker _et al._, 1984a; Dewhurst, 1984), and these increases in trace amine levels have been proposed to contribute in part to the therapeutic efficacy of this class of drugs (Boulton, 1984; Dewhurst, 1984). In addition to the affective disorders, PEA has been implicated in the etiology of schizophrenia, migraine, phenylketonuria, Parkinsonism and tardive dyskinesia (for reviews see Wyatt et al., 1977; Mosnaim and Wolf, 1978; Wolf and Mosnaim, 1985).

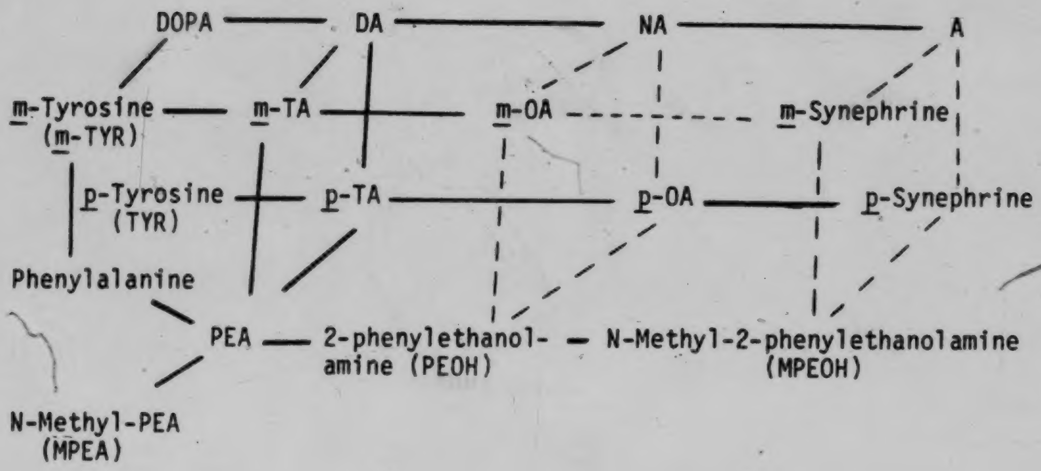
J.1.1 Metabolic Relationship of PEA to Other Endogenous Amines.

Administration of PEA, DA or DOPA to rats has been reported to result in the formation of tyramines (Brandau and Axelrod, 1972; Boulton and Dyck, 1974, 1974a; Dyck and Boulton, 1975). Boulton (1976b) proposed the metabolic interrelationships between phenylalkylamines in mammalian brain shown in Fig. 9.

A glucuronide metabolite (presumably a N-glucuronide) has also been suggested for PEA (Inwang et al., 1973), but the major catabolic pathway is deamination by MAO to form phenylacetic acid which is excreted in urine mainly as phenylacetylglutamate.

J.1.2 Interactions of PEA with putative neurotransmitter amines

Administration of PEA to rodents results in depletion of central and peripheral catecholaminergic stores (Jonsson and Grobecker, 1966; Fuxe et al., 1967; Jackson and Smythe, 1973). Subcellular fractionation studies revealed that preferential depletion occurred in a particulate fraction (Jonsson et al., 1966). Behavioral effects of PEA have been reported to be blocked by pretreatment of animals with reserpine and tyrosine hydroxylase inhibitors (which both cause depletion of catecholamine levels), and, since these behavioral effects resemble those of amphetamine, Fuxe et al. (1967) proposed similar mechanisms in the


DOPA
DA
NA
A
m-Tyrosine (m-TYR)
m-TA
m-OA
m-Synephrine
p-Tyrosine (TYR)
p-TA
p-OA
p-Synephrine
Phenylalanine
PEA
2-phenylethanolamine (PEOH)
N-Methyl-2-phenylethanolamine (MPEOH)
N-Methyl-PEA (MPEA)

[______: established ; ------: proposed]

Fig. 9. Metabolic interrelationships between phenylalkylamines in mammalian brain (adapted from Boulton, 1976b).

behavioral actions of PEA and amphetamine; however iontophoretic studies of Giardina *et al.* (1973) contradict this. Recently, by employing mass fragmentographic techniques, Chuang *et al.* (1982) reported specific increases in brain PEA levels following amphetamine administration but not after methamphetamine even though both drugs mediate their actions through catecholamine release. This is in contradiction to an earlier report by Danielson *et al.* (1976) indicating no changes in PEA levels after amphetamine treatment (both acute and chronic administration). In attention deficit disorder patients receiving d-amphetamine, urinary PEA excretion was significantly increased, thus lending credence to the earlier suggestion that amphetamine might mediate some of its behavioral effects through specific increases in PEA (Zametkin *et al.*, 1984). Studies of Raiteri *et al.* (1977), Baker *et al.* (1978), Karoum *et al.* (1982), McQuade and Wood (1983), Philips and Robson (1983), Dyck (1983, 1984), Nielsen *et al.* (1983) and Lundberg *et al.* (1986) demonstrate the catecholamine-releasing properties of PEA (for a detailed discussion refer to chapter IV). Based on early experiments, interactions with serotoninergic systems appeared to be ~~limited (~~Jackson and Smythe, 1973); however, several recent reports indicate that some important behavioral effects of PEA may be mediated through such mechanisms (Sloviter, 1980; Dourish, 1981, 1982). Stereotypies produced by amphetamine and PEA in experimental animals have been shown to be associated with disaggregation of polyribosomes and inhibition of protein synthesis *in vivo* (Widelitz, 1979). Using methysergide (a 5-HT blocking agent), pimozide (a postsynaptic DA receptor blocker) and diethyldithiocarbamate (a NA synthesis inhibitor), Widelitz and Hable (1981) demonstrated that amphetamine induces behavioral effects and polyribosomal disaggregation through release and activity of DA, while PEA acts through 5-HT and, to a lesser extent, through NA and DA.

J.1.3 Binding sites for PEA

Hauger *et al.* (1982) reported specific [^{3}H]-PEA binding in rat brain. The binding exhibited a B_{max} of 1078 fmol/mg protein, with highest specific binding in the hypothalamus and striatum, regions reported to contain highest amounts of PEA (Boulton, 1976a). These binding sites appear to be distinct from those for other known neurotransmitters and for [^{3}H]-amphetamine. Furthermore, none of the currently employed neuroleptics, tricyclic antidepressants, or benzodiazepine tranquilizers was capable of displacing [^{3}H]-PEA from its binding sites. Since this early report, there have been no further reports from other laboratories substantiating or contradicting the claim. It is possible that many behavioral and neurochemical effects of PEA might be mediated through these receptor sites, and further work is required to elucidate this aspect.

J.2 Tryptamine

The presence of T in various tissues of mammals has been reported and confirmed in recent years, but the actual values reported for various organs differ significantly depending upon the analytical technique employed (see Jones, 1982 for a review). Saavedra and Axelrod (1972), utilizing a radioenzymatic technique, reported T levels in the range of 14-51 ng/g in peripheral tissues of rat and about 20 ng/g in brain. Snodgrass and Horn (1973) used a radiochemical assay that employed reaction with [^{3}H]-dansyl chloride, and reported rat brain levels of 70 ng/g. Philips et al. (1974) utilized a highly specific thin layer chromatography-high resolution mass spectrometry (TLC-HRMS) technique which yielded brain levels of less than 0.5 ng/g and levels of 0.4-8 ng/g in peripheral tissues; similar values were reported by Warsh et al. (1977), Artigas and Gelpi (1979) and Artigas et al. (1984) using combined gas chromatography with mass spectrometry (GC-MS). Recently, Larson and Dalo (1986) employed HPLC with fluorescence detection and reported levels around 0.6 ng/g.

As pointed out earlier, pretreatment with MAO inhibitors causes marked increases in tissue T levels (Boulton et al., 1973; Philips and Boulton, 1979; Philips et al., 1980; Dewhurst, 1984; Baker et al., 1984a) relative to those seen with NA, DA and 5-HT after administration of the same drugs. L-Trytophan, iproniazid and p-chlorophenylalanine (a tryptophan hydroxylase inhibitor) each produced moderate increases in brain T concentrations, whereas combined administration of the above drugs produced much larger increases; administration of a central AADC inhibitor decreased brain T levels moderately and reserpine treatment did not change T levels (Saavedra and Axelrod, 1973). Employing high resolution mass spectrometric techniques, Juorio and Durden (1984) reported that T formation is a significant but not major pathway for tryptophan metabolism. Sloan et al. (1975), using a fluorometric procedure, studied effects of a number of drugs on the levels of T in various species; they observed that rat brain contained lower T levels (21 ng/g) than did dog and cat brain and that MAO inhibitors increased T levels while reserpine was without effect. Juorio and Durden (1984) reported decreases in 5-HT and 5-HIAA levels in mouse brain and rat spinal cord following treatment with p-chlorophenylalanine, but could detect no change in levels of T, whereas the reports of Saavedra and Axelrod (1973) cited above and that of Marsden and Curzon (1974) indicated increases in brain T levels after such treatment. But it must be kept in mind that the control levels found by these two groups are much higher than those found by Juorio and Durden (using a mass spectrometric method). Marsden and Curzon (1974) also reported the lack of depletion of T levels in reserpine-treated rats, but observed decreased levels of T following serotoninergic and dopaminergic lesions, indicating synthesis of T in these two neural tracts. These results are at variance with those of Juorio and Greenshaw (1985) who reported that concentrations of T were independent of the integrity of 5-HT neuronal system. A recent report by the same workers indicated that T levels were dependent on the integrity of the substantia nigra, suggesting the possible existence of T-containing neurons in the nigrostriatal system (Juorio and Greenshaw, 1986). The controversial issue of vesicular (Snodgrass and Horn, 1973; Lemberger *et al.*, 1981;) and nonvesicular storage of T (Saavedra and Axelrod, 1973; Marsden and Curzon, 1974; Sloan *et al.*, 1975) also appears to be due to methodological problems, although using the highly sensitive TLC-HRMS technique, Juorio (1982) reported an increase in mouse striatal T after reserpine, further complicating the issue.

J.2.1 Metabolism of T

Following an intraventricular injection of T in control rats, the only metabolites of T isolated were indole-3-acetic acid (IAA) and indoleacetaldehyde; the proportion of aldehyde significantly increased if sodium thiosulfate was included in isolation procedures, indicating that a significant amount of acid is formed during the isolation procedure (Wu and Boulton, 1973). Additional metabolites found in small proportions were N-methyl-T, N,N-dimethyl-T and 6-hydroxy-T (Jepson *et al.*, 1962; Lemberger *et al.*, 1971). Intraventricularly injected T disappeared from brain quite rapidly, indicating rapid turnover (Wu and Boulton, 1973). Enzyme specificity of T for oxidation by MAO appears to be controversial. Houslay and Tipton (1974) considered T to be solely metabolized by MAO-B, while Neff and Yang (1974) and Durden and Philips (1980) indicated oxidation by both MAO-A and -B; marked increases in brain T levels after administration of the MAO-A-selective inhibitor clorgyline but not after the MAO-B-selective inhibitor deprenyl (Philips and Boulton, 1979) suggest MAO-A-mediated oxidaton of T. *In vitro* studies indicated that the reversible MAO-A inhibitor harmaline was more potent at inhibiting T deamination than was PAR (MAO-B specific inhibitor at low doses) (Fuller, 1972). In humans, the reversible MAO-A inhibitor CGP 11 305A caused greater urinary excretion of T than did (-)-deprenyl (Bieck *et al.*, 1984) and clorgyline caused significantly greater reduction in rat brain T binding (B_{max}) than did deprenyl (Wood *et al.*, 1985) upon chronic administration, pointing to predominant deamination of T by MAO-A. Kinetic measurements (Durden and Philips, 1980) indicated that the half-life of T was 1 min, in contrast to 77 min for 5-HT. While the endogenous concentration in rat brain of T is less than 0.01% of that of 5-HT, its synthesis rate was shown to be about 10% of that of 5-HT. A high rate constant of oxidation (Durden and Philips, 1980) and high affinity for MAO (Houslay and Tipton, 1974) suggest a cytoplasmic rather than a vesicular storage site, although this remains a matter of controversy.

J.2.2 Interactions of T with putative neurotransmitters.

The ability of T to inhibit active reuptake of 5-HT has been demonstrated in rat hypothalamus homogenates (Horn, 1973) and rat striatal prisms (Baker et al., 1977; Baker et al., 1980c). Born et al. (1972) and Airaksinen et al. (1980) have observed 5-HT uptake inhibition in human platelets by T, and Born et al. (1972) proposed the inhibition to be of a competitive nature. In contrast, Ross and Ask (1980) have suggested that in rat hypothalamus and in mouse whole brain T is unlikely to interact with the 5-HT carrier system. Baker et al. (1980c) observed moderate inhibition of DA uptake by T while Ennis et al. (1981) could not confirm this. Baker et al. (1977, 1980c) reported potent 5-HT release and moderate DA release in rat striatum by T. Effects of T on release of ACh at the frog neuromuscular junction and the release of glutamate at the lobster neuromuscular junction have also been observed (Friedman, 1979; Friedman et al., 1981).

Because of the structural similarity of the two amines, interactions of T with 5-HT systems have been studied extensively. Tryptamine can displace the postsynaptic (presumably) binding of 5-HT in brain tissue (Bennett and Snyder, 1976; Whitaker and Seeman, 1978; Seeman et al., 1980). Because of low endogenous levels of T, the degree of antagonism might be expected to be low, although the physiological relevance of such antagonism is not clear. Single cell recording studies in rat cortex (Jones and Boulton, 1980) indicated that application of low amounts of T which do not have any influence per se on firing rates of cells consistently abolished excitatory responses to iontophoretically applied 5-HT; often the excitation evoked by 5-HT was replaced by depression in the presence of T. Rats anesthetized with urethane exhibit an automatic swallowing reflex which appears to be dependent upon tonic activity of central 5-HT in that 5-HT-releasing agents and uptake inhibitors such as p-chloroamphetamine and fluoxetine enhance this reflex; this enhancement can be blocked by small doses of intravenously administered T (Jones, 1981). Modulation of 5-HT-mediated responses by T has also been shown at the behavioral level. Jones (1981) observed that T antagonized 5-hydroxytryptophan (5-HTP)-induced head twitches in mice while there was a concurrent enhancement of other behavioral signs (hind limb abduction, tremor, head weaving, etc.). Dewhurst and Marley (1965) and Marley and Nistico (1975) examined the effect of T on behavior in the immature and adult fowl. Intraventricular injection of T in the adult fowl caused rapid head jerking, repetitive preening, vocalization, pecking, wing abduction and neck extension; similar gross behavioral changes were found following intravenous injection into the immature fowl (Dewhurst and Marley, 1965; Jones, 1981) and these actions were blocked by pretreatment with methysergide (Dewhurst and Marley, 1965).

J.2.3 Tryptamine receptors.

Cascio and Kellar (1983) reported the presence of specific binding sites for T in brain although T receptors had been distinguished from 5-HT receptors in the periphery as early as 1957 (Wooley and Shaw, 1957) and Dewhurst (1968) had previously proposed the existence of T receptors in the CNS. Brain T binding sites were found to be distributed widely throughout the brain, with highest density occurring in cerebral cortex, striatum and hippocampus; similar binding sites were observed in human cortex. Subcellular fractionation studies revealed the presence of binding sites mainly in the synaptosomal fraction. Competition studies indicated that T binding sites were distinct from 5-HT receptors. Tetrahydro-β-carboline, quipazine, PEA, amphetamine, p-chloroamphetamine and methamphetamine inhibited [^{3}H]-T binding (Cascio and Kellar, 1983). Wood et al. (1984) further characterized the binding site and reported on its stereospecificity. They also suggested that the T binding site may represent the T receptor in brain. Compounds derived from T such as tryptolines appear to be potent displacers of [^{3}H]-T binding (Wood et al., 1984). Bruning and Rommelspacher (1984) characterized T binding sites in different organs of rat and Charlton et al. (1985) reported that kynuramine had high affinity for the [^{3}H]-T binding site. Perry (1986), employing in vitro autoradiographic techniques, examined the distribution of [^{3}H]-T binding sites in rat brain. He observed a concentration of T binding sites in forebrain and limbic structures, with the highest number of sites in choroid plexus and interpeduncular nucleus. A binding site for [^{3}H]-T in choroid plexus (termed T-2) was pharmacologically distinct from the rest of the brain sites (termed T-1) in that kynuramine and several other compounds inhibited [^{3}H]-T binding at the T-1 site but not at the T-2 site. [^{3}H]-5-Hydroxytryptamine also exhibited a binding site in the choroid plexus but it was distinct from the T-2 site of T. Using quantitative autoradiography, Kaulen et al. (1986) studied the distribution of [^{3}H]-T binding sites in rat brain and observed the β-carbolines harmine and harmaline to be potent displacers, while 5-HT as well as several 5-HT receptor antagonists such as methysergide and metergoline were observed to be weak displacers of [^{3}H]-T binding. The pattern of distribution and displacement characteristics indicated that [^{3}H]-T binding sites were uniquely distinct from 5-HT$_1$, 5-HT$_2$ and [^{3}H]-imipramine binding sites (Kaulen et al., 1986).

McCormack *et al.* (1986) employed quantitative autoradiography to study the distribution of [^{3}H]-T binding sites in numerous brain regions of rat and dog and observed highest densities of binding in more rostral portions of the brain. Altar *et al.* (1986) reported that [^{3}H]-T binds to rat brain regions with up to 58% specificity (as determined with 10 M T as a displacer), with greatest binding in nucleus accumbens and claustrum. *p*-Methoxyphenylpropylamine, harmaline and 5-methyltryptamine displaced [^{3}H]-T binding in the nanomolar range, whereas 5-HT caused displacement in the micromolar range (Altar *et al.*, 1986). Chronic administration of PAR to rats was reported to decrease [^{3}H]-T binding in cerebral cortex, hippocampus, striatum and hypothalamus whereas neurotoxins such as 6-hydroxydopamine and 5,7-dihydroxytryptamine did not affect [^{3}H]-T binding, indicating respectively that changes in T metabolism alter the [^{3}H]-T binding and that the locations of binding sites were distinct from 5-HT and DA neurons (Cascio and Kellar, 1986). At the present time there are no agonists or antagonists specific for [^{3}H]-T receptors (binding sites), although several compounds exhibit affinity for them. Thus the functional significance of T binding sites remains unresolved.

Because of the possible modulator or transmitter function of T in brain, disturbances in synthesis and metabolism of T could contribute to the pathophysiology of psychiatric disorders. Significant correlations between increased urinary T output and exacerbated symptoms in schizophrenia (Brune and Himwhich, 1961; Herkert and Keup, 1969) were noted, and urinary T output has been reported to be altered in the general psychiatric population (Slingsby and Boulton, 1976). Coppen et al. (1965) observed decreased urinary T output in depressed patients, with no correlation with indoleacetic acid (IAA), the major metabolite of T. Kuehl et al. (1968) indicated that some Parkinsonian patients may excrete abnormally high amounts of T in their urine. However, Anderson et al. (1984) recently studied central T turnover by measuring IAA levels in cerebrospinal fluid (CSF) of normals, schizophrenics, anorectics, manics and depressives and observed no significant changes in T turnover in these disorders.

From the brief review presented above it seems likely that the trace amines (PEA and T) may play a role in the etiology of various affective and neurological disorders. To adequately study the functional significance of PEA and T, it should be possible to selectively alter their concentrations without affecting those of other neurotransmitters and modulators. Nonselective increases in these biogenic amines following the administration of MAO inhibitors negate the use of these drugs to elucidate the role of trace amines. Rapid metabolism of the trace amines themselves preclude their use alone to selectively increase and sustain brain PEA and T levels. In the investigation reported in this thesis, a number of N-alkylated analogs of PEA and T were examined for their abilities to increase and sustain the parent amine levels. The neurochemical consequences of such an alteration in brain trace amines with the concomitant presence of their prodrugs have also been examined by measuring the levels of putative neurotransmitter amines and their metabolites. The results of this work are presented in subsequent sections.

K. Analytical Techniques

During the course of these investigations levels of arylalkylamines and their prodrugs were measured in rat brain, blood and liver by analytical techniques that utilized gas chromatography with electron-capture detection (GC-ECD). The levels of the putative neurotransmitter amines NA, DA and 5-HT and the acid metabolites homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) were quantitated using a modification of a previously reported high-pressure liquid chromatography technique with electrochemical detection (HPLC-EC) (Saller and Salama, 1984). Brief accounts of these techniques are presented in subsequent sections.

K.1 Gas chromatography

Gas chromatography (GC) is a vapour phase separation technique that is used for both quantitative and qualitative purposes and it is in the quantitative mode that GC has been used most widely in neurochemistry. In common with many liquid chromatographic techniques, GC employs stationary phases and mobile phases and separation is achieved by the relative partition of various components of a mixture between these two phases. In GC, the stationary phase is generally a high boiling point organic liquid which is either polar or nonpolar depending upon the compounds to be separated on it. Usually the stationary phase is coated onto particles of a support material and these are packed into columns which are then housed in a gas chromatographic oven. Numerous inert gases such as helium, argon, nitrogen and hydrogen have been used in GC as carrier gases (mobile phases). In liquid chromatography, polarity of the mobile phase is altered to elute components in a mixture that are separated by differential partition between stationary and mobile phases and in GC oven temperature programming is employed to effect the same. The vapour carrier gas sweeps the components through the column as they separate on the basis of vapour phase characteristics and the polarity of the column. The oven temperature range used is governed by the stability of the stationary phase and that of the detector as well as the volatility of the components to be separated. Isothermal (oven temperature held constant) separations may be used if temperature programming fails to resolve closely eluting peaks.

During the course of the present investigation all the gas chromatographic separations were performed on high resolution fused silica capillary columns. Such columns offer the resolution and degree of separation necessary for trace analysis. Extensive reviews on types of gas chromatographic columns available and their application to neurochemistry, pharmacology and clinical biochemistry are available (Walker *et al*., 1977; Jennings, 1980; Freeman, 1981; Baker *et al*., 1982; Coutts *et al*., 1985; Jaeger, 1985).

K.1.1 Detectors for GC

The electron-capture detector (ECD) and the nitrogen-phosphorous detector (NPD) are the two most widely employed detectors in analytical neurochemistry, although flame ionization and thermal conductivity detectors are also used occasionally. For reviews on GC detectors see Walker et al. (1977), Hall (1978), Baker *et al*. (1981,1982), Coutts and Baker (1982), and Coutts *et al*. (1985). The discussion here will be limited to the ECD and to the use of the mass spectrometer as a highly specialized detector.

K.1.1.1 Electron-capture detector. Lovelock and Lipsky (1960) first introduced the ECD, and since then it has undergone several variations to attain the present day's selectivity and sensitivity. The detector comprises a source of continuous β-particles such as ^3H or ^{63}Ni that will ionize the carrier gas molecules, generating a number of secondary electrons. Mutual collisions of these secondary electrons generate low-energy thermal electrons. These thermal electrons are collected at a positively charged plate (anode) and constitute the standing current (baseline). The introduction of a sample component from the GC column which is capable of attracting (or capturing) the secondary electrons decreases the number of electrons collected by the anode, hence producing a decrease in standing current. The decrease in current is proportional to the concentration of the substance capturing the electrons. The disturbance in baseline current, after suitable amplification and sign change, is recorded as a positive peak on the recorder.

The ECD is commonly operated in either constant voltage or pulsed voltage mode which differ primarily in the method of thermal electron collection at the anode. In the constant voltage mode, a fixed voltage is applied to the anode which constantly collects thermal electrons. As large amounts of electrophoric component elute from the GC column it may deplete the thermal electrons and become negatively charged and compete with thermal electrons for collection at the anode, thereby decreasing the linearity of response and sensitivity. One way of overcoming the problem is to apply short pulses (duration of 1 μsec) of voltage for relatively short time intervals (5 to 150 μsec) to the anode. By careful adjustment of pulse width and pulse frequency one can extend the dynamic range of the ECD from 200 (of constant voltage mode) to 1000 (Baker *et al.*, 1981). A third mode is the pulsed voltage and constant current method as introduced by Maggs *et al.* (1971); this type of ECD has picomole sensitivity with a dynamic range extending up to 10,000. Most ECDs employ ^{63}Ni as a β-emitter because of the longer half-life (92 years) relative to that of ^{3}H. The activities of the β-sources vary from 10-1000 mCi: in general, an increase in the β-source activity leads to an increase in the sensitivity and the linear dynamic response range, hence ^{3}H as a β-source provides greater sensitivity than ^{63}Ni.

K.2 Gas chromatography-mass spectrometry (GC-MS)

Mass spectrometry (MS) is a unique spectrometric technique capable of structural elucidation (determination of functional groups and exact molecular weight) as well as quantitation. In the present investigation, combined GC-MS was used for characterization of all derivatives formed during development of novel methods for analysis of the amines and analogs of interest. Interfacing MS with GC results in a powerful tool capable of separation, characterization and quantitation of complex mixtures. In this form, the mass spectrometer forms a highly selective and sensitive detector, albeit a costly one. The effluent from the GC is enriched in content of the analyte by removing carrier gas (or carrier gas + makeup gas) before passing the sample into the mass spectrometer. In the mass spectrometer the analyte undergoes ionization either due to bombardment with high energy electrons (electron impact, EI) or due to collisions with an ionized reactant gas (chemical ionization, CI) such as methane, isobutane or ammonia, to generate positive, negative and neutral fragments; a total ion current (TIC) profile of either positive ions or negative ions can be recorded at the detector. In the single-ion-monitoring (SIM) mode, one ion fragment unique for the analyte can be monitored, thus vastly improving the sensitivty of the detector. Use of the SIM mode is often referred to as mass fragmentography. In this mode, GC-MS is widely used for quantitative purposes, while in the multiple ion monitoring mode GC-MS is employed to confirm the presence of a compound in a mixture. In the EI-MS mode, because of the high energy electron bombardment, ion fragmentation is quite extensive and often a molecular ion may not be present and other fragments may not be structurally unique for the analyte. In CI-MS, milder ionization usually generates fewer fragments that carry the charge (positive or negative). These have high masses and are often structurally unique for the analyte, especially the quasimolecular ion MH^+. Thus CI-MS-SIM mode operation may offer greater sensitivity for quantitative analysis than does EI-MS-SIM.

The application of GC-MS to neurochemistry has been the subject of a number of recent reviews (Gelpi, 1982; Faull and Barchas, 1983a; Durden, 1985; Karoum, 1983,1985).

K.3 Derivatization for GC-ECD

During the course of present work GC-ECD was employed to analyze bioactive trace amines and their prodrugs and TCP and its analogs in body fluids and tissues of experimental animals and some patient urine samples, hence the discussion of derivatization procedures is limited to their applications to GC-ECD. Extensive reviews of derivatization techniques are available (Ahuja, 1976; Blau and King, 1978; Knapp, 1979; Baker et al., 1981; Faull and Barchas, 1983b; Coutts et al., 1985). The choice of derivatizating agents for analysis is governed by several factors:

1. type of derivative formed (single or multiple) and its stability during handling and storage (silyl and alkyl boronates are highly moisture-sensitive).
2. nature of the derivatizing agent, its stability during storage (silyl derivatizing agents are sensitive to moisture).
3. conditions in which the derivatization reaction has to be conducted (aqueous or anhydrous) which in turn dictate sample preparation.
4. degree of volatility imparted by derivatization (fluorinating agents do not generally increase boiling point whereas other halogenated reagents markedly increase boiling point of the derivative).
5. degree of separation (empirical predictions are often difficult to make, and separation is a function of GC conditions in their entirety, e.g., type of column, length of column, temperature programming, etc.)
6. further use of the derivatized analyte (i.e. whether derivative is to be employed for GC-MS-SIM or not)
7. response on ECD.

During the course of investigations described in this thesis pentafluorobenzoyl chloride (PFBC) and pentafluorobenzenesulfonyl chloride (PFBSC) have been extensively used to derivatize arylalkylamines and their prodrugs, and detailed techniques are presented in Chapter II.

Acid chlorides as acylating agents form an important group of derivatizing agents because of their extreme reactivity towards phenolic

Table 7. Representative examples of GC-ECD analysis using PFBC and PFBSC as derivatizing agents.

Derivatizing Agent	Compound(s)	Functional Group Derivatized	Derivatization Conditions [aqueous (Aq), nonaqueous (NAq)]	References
PFBC	Amphetamine Phenmetrazine	amino	NAq	Angaard and Hankey (1969)
	Amphetamine and Ephedrines	amino	NAq	Wilkinson (1970)
	Clonidine	imino and imidazolidine	Aq	Nazarali *et al.* (1986b)
	Amphetamine	amino	NAq	Midha *et al.* (1979a)
	Fenfluramine Norfenfluramine	amino	NAq	Midha *et al.* (1979b)
	Amphetamine, Chloramphentamine, Cyclopentamine, Ethylamphetamine, Fencamfamine, Fenfluramine, other doping agents, and TCP	amino	Aq	Delbeke *et al.* (1983)
	NA, DA	amino, phenolic alcoholic	NAq	Bock and Weser (1981)
	Amphetamine	amino	Aq	Cristofoli *et al.* (1982)

Derivatizing Agent	Compound(s)	Functional Group Derivatized	Derivatization Conditions [aqueous (Aq), nonaquous (NAq)]	References
PFBC	Amphetamine, *p*-OH-amphetamine, *N*-alkylated amphetamines	amino, phenolic	Aq	Nazarali *et al.* (1984)
	PEA	amino	NAq	Moffat *et al*. (1972)
	PEA	amino	Aq	Nazarali *et al.* (1986a)
	Methoxyphenamine and its ring hydroxylated metabolites	amino and phenolic	Aq	Roy *et al.* (1984)
	PEA, T, *m*- and *p*-TA, HA, MeHA, normetanephine, 5-HT	amino and phenolic	Aq	Wong (1985)
	PEA and N-alkylated analogs	amino	Aq	present investigation and Coutts *et al.* (1985)
	PLZ	hydrazine	Aq	Rao *et al.* (1986c)
	Tricyclic antidepressants	amino and phenolic	Aq	Baker *et al.* (1986c)

Derivatizing Agent	Compound(s)	Functional Group Derivatized	Derivatization Conditions [aqueous (Aq), nonaquous (NAq)]	References
PFBSC	PEA	amino	Aq	Baker *et al.* (1986a)
	TCP	amino	Aq	Rao *et al.* (1986a)
	PEA and N-alkylated analogs	amino	Aq	present investigation
	T and N-cyanoethyl-T	amino (aliphatic but not indolic)	Aq	present investigation
	N-alkylated analog of TCP and ring substituted TCP analogs	amino	Aq	present investigation
	Tricyclic antidepressants	amino	Aq	Baker *et al.* (1986c)
	Tyrosylpeptides and nucleic acid pyrimidine bases	amino and phenolic	NAq	Sentissi *et al.* (1984) Nazareth *et al.* (1984)

hydroxylic and amino groups under both aqueous and anhydrous conditions. Table 7 shows some representative applications of PFBC and PFBSC as derivatizing agents for various types of compounds.

From Table 7 it is evident that the earlier techniques have employed primarily nonaqueous derivatization conditions which require the analytes to be in an organic solvent or in a dry form. Extraction of trace amines and drug molecules having multiple functional groups from biological samples has not always been satisfactory. It would be advantageous to combine extraction with a derivatization step to simplify the assay procedures. During the course of the investigations reported in this thesis, both PFBC and PFBSC have been employed to effect extractive derivatization under aqueous conditions. Another approach with which to increase extraction efficiency of polar organic molecules containing amino and/or phenolic functional groups into organic solvents is to convert them into nonpolar derivatives under aqueous conditions by reaction with acetic anhydride followed by extraction; this must then be followed by reaction with a reagent that will provide electron-capturing groups if GC-ECD is to be used for analysis. The applicability of such a technique has been extensively reviewed in Baker _et al._ (1981, 1982) and Coutts _et al._ (1985).

K.4 High pressure liquid chromatography (HPLC)

Conventional column chromatographic techniques employed gravity-fed open column adsorbents such as silica gel and alumina with fairly large particle size, resulting in appreciable band spreading due to differential mass transfer. Development of pellicular adsorbents (30-45 μm particle size) resulted in more efficient separation with minimized band spreading. With the development of efficient columns and low dead-volume detectors, HPLC became a routine technique for the analysis of several bioactive amines. This technique combined with electrochemical detection (HPLC-EC) is particularly well suited for the analysis of catecholamines, indoleamines and their metabolites. Present day HPLC columns for reverse-phase separation are usually made up of silica chemically bonded to either octadecylsilane (C_{18}) or C_{22} hydrocarbon available in a particle size range of 5-10 μm. Goldberg (1982) compared different reverse-phase HPLC columns that are commercially available. The basic mechanism of separation involves differential mass transfer of analyte between stationary phase and mobile phase. Depending upon the nature of the mobile phase, HPLC can be divided into two types: (1) Normal phase HPLC. In this technique the mobile phase is nonpolar (usually organic) as is the stationary phase. (2) Reversed-phase HPLC (RP-HPLC), in which a polar (aqueous) mobile phase is employed. The type of detection system and the nature of analytes dictate the nature of mobile phase that can be employed; for electrochemical detection, normal phase HPLC is unsuitable because of low oxidizability of neurochemicals in organic solvents. For most HPLC-EC applications, aqueous mobile phases with reasonable ionic content (usually millimolar concentrations) are widely used. Many of the catecholamines, indoleamines and their metabolites are either amphoteric or too polar and elute very quickly with the mobile phase; resolution of these compounds requires modification of RP-HPLC. Majors (1980) identified six techniques in RP-HPLC, namely (1) regular, (2) ionization control, (3) ion suppression, (4) ion pair, (5) secondary equalibrium and (6) non-aqueous. Among these six combinations of techniques (2)-(4) are in use in RP-HPLC to achieve an optimum degree of separation of mixtures of catecholamines, indoleamines and their metabolites. Recognition of the fact that modification of the polarity of the analytes relative to the mobile phase affects the mass transfer of analytes (i.e. separation) led to the employment of aqueous buffers with (a) controlled pH to limit the analyte's ionization, (b) organic modifiers to reduce surface tension between the adsorbent and the mobile phase and to change the net polarity of the mobile phase, and (c) ion pairing agents to reduce the analyte's polarity. Buffers are prepared to contain the above complementary factors either alone or in combination to suit the analysis requirements.

Control of pH can be achieved by proper selection of buffer reagents; in general, acetate, phosphate or citrate buffers are employed in RP-HPLC in the pH range of 2-8. This pH range offers the best stability for the adsorbent (HPLC column). Efficiency of separation and column stability should be kept in mind when selecting an appropriate buffer system. Citrate buffer has been reported to cause permanent deterioration of octadecylsilane (ODS) columns (Freed and Asmus, 1979), whereas acetate buffers are reported to give low column efficiency, possibly due to the formation of nonpolar complexes with analyte molecules (Brown and Krstulovic, 1979). In potassium-containing buffers, potassium has been reported to form an insoluble complex with sodium dodecylsulfate, a commonly employed ion-pairing agent (Crombeen _et_ _al._, 1978).

Addition of organic modifiers such as acetonitrile, methanol and tetrahydrofuran alter the solvation power of the solvent, reduce surface tension between mobile and stationary phases and decrease the polarity of the eluents (Berendsen and De Galan, 1980), although the exact mechanisms of their influence are not clear. Increasing concentrations of organic modifiers generally decrease the retention time of polar analytes on a HPLC column.

The pH value of the buffer determines the extent of ionization or ion suppression of biogenic amines. At acidic pH values, the amino groups of catecholamines, indoleamines and their metabolites will be completely ionized, whereas in alkaline pH the acidic functional groups will be ionized. Addition of an anionic soap (e.g. alkyl sulfates) to the acidic mobile phases results in ion-pair formation with the amino groups of the neurochemicals. In the absence of such an ion-pairing agent, sufficient separation is difficult to achieve. Commercially available ion-pairing agents differ in their alkyl chain length; the longer the alkyl chain the longer would be the retention time of the analyte undergoing ion-pairing (Deelder et al., 1979; Melander and Horvath, 1980; Knox and Hartwick, 1981) and the higher the concentration of ion-pairing agent, the longer the retention time (Horvath et al., 1977; Deelder et al., 1979; Knox and Hartwick, 1981; Kontur et al., 1984). Because of longer equilibration times required between the stationary phase and the ion-pairing agent (Michaud et al., 1981), predictions of retention times are difficult to make. Under slightly alkaline pH conditions, alkyl boronates could be employed as catechol-specific ion-pairing agents (Joseph, 1985). Normally the ion-pairing agents are added at a concentration of 5 to 10 mM to the mobile phase, depending upon the degree of resolution required.

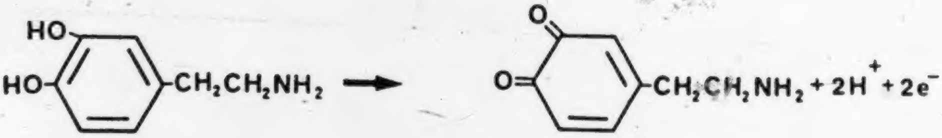


Fig. 10 Electrochemical oxidation of dopamine (DA)

Detailed discussions regarding the use of ion-pairing agents in RP-HPLC may be found in Horvath et al. (1977), Deelder et al. (1979), Melander and Horvath (1980), and Knox and Hartwick (1981).

Among the available detectors, the electrochemical detector is most suitable for the analysis of catecholamines, indoleamines and their metabolites. Compounds containing phenolic groups or the indole structure can be oxidized. A typical oxidation reaction for DA is presented in Fig. 10.

Such reactions can be brought about electrochemically since they only require electron transfer processes, and the electrochemical procedures are based on the measurement of the current (either generated or consumed) associated with a redox reaction of a electrochemically active analyte on the surface of an electrode; the change in current is proportional to the concentration of analyte causing such changes. If ionic strength, pH and temperature of the buffer are kept constant, quantitative electrochemical detection is possible. Under identical reaction conditions each electrochemically active analyte exhibits a characteristic "half wave potential", a measure of feasibility of electrochemical reactions, and, with a proper selection of oxidation potential, certain compounds can be oxidized while excluding others.

Commercially employed electrochemical detectors can be divided into two categories according to cell design and electrolytic efficiency: amperometric and coulometric. All amperometric detectors are compact in size, with a cell surface area < 0.5 cm^2, and have an electrolytic efficiency around 1-10%. Because of the small surface area (the dead volumes are typically less than 10 μl), the background—current is usually low and the flow fluctuations are minimal. In contrast the coulometric detectors have greater electrode surface area, which leads to large dead volumes in the electroanalytical cell and considerable baseline noise. The electrolytic efficiency approaches nearly 100%, which means a marked increase in sensitivity compared to an amperometric detector. In recent years coulometric detectors with <5 μl total cell volume and low background current noise have been made available. Electrochemically active analytes enter the electrochemical cell continuously as they are separated from the column, are oxidized or reduced at the surface of working electrode and removed from the cell after contact with reference and auxiliary electrodes. Glassy carbon electrodes are widely employed as the working electrodes because of their robustness, whereas Ag/AgCl electrodes and stainless steel or platinum electrodes are employed as reference and auxilliary electrodes respectively. Following are some compounds of neurochemical interest that can be analysed by HPLC-EC.

I. Tyrosine metabolites:

tyrosine (TYR), OA, TA, synephrine, hordenine, DA, α-methyldopamine, epinine, A, NA, aldomet, NME, metanephrine

II. Tryptophan metabolites:

tryptophan (TRP), 5-HTP, 5-HT, 5-HIAA, bufotenin, kynuramine, kynurenic acid, xanthenuric acid

III. Phenylpyruvic acids

4-hydroxyphenylpyruvic acid and 4-hydroxy-3-methyoxyphenylpyruvic acid

IV. Acidic and Neutral Metabolites

HVA, protocatechuic acid, 4-hydroxymandelic acid, 3,4-dihydroxymandelic acid (DOMA), vanillylmandelic acid (VMA), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), 3,4-dihydroxyphenylethylene glycol (DHPG), 3,4-dihydroxyphenylethanol (DOPET), 3-methoxy-4-hydroxyphenylethanol (MOPET)

The catecholamines, indoleamines and their metabolites can be oxidized (depending upon the ionic strength of mobile phase) at an applied voltage of 0.75-0.9 V against the reference electrode, whereas amino acids such as TRP and TYR are typically oxidized at somewhat higher voltages of 1.0-1.1 V.

Compared to GC, HPLC-EC offers many advantages, namely: (1) HPLC is a liquid phase separation technique, hence there is often no need to extract analytes into organic solvents and perform derivatization reactions; however derivatization is also applied in HPLC for some amines and amino acids that are difficult to detect by HPLC-EC techniques; (2) if proper guard columns are employed, sample preparation is virtually straightforward, and direct injection of brain or tissue homogenates is practiced; (3) HPLC-EC is ideally suited for analytes with suitable redox properties and which are thermolabile; (4) for many catecholamines, indoleamines and their metabolites, HPLC-EC offers sufficient sensitivity for regional analysis in brain tissue (tissue weights <50 mg), and such sensitivities are equal to those obtained with GC-MS; and (5) although GC techniques allow structural elucidation when combined with MS and afford unique sensitivity in the GC-MS-SIM mode, often mass spectrometric facilities are not within the financial reach of small laboratories. Recent developments have led to the manufacture of HPLC-MS on a commercial scale (albeit expensive) which could be employed for structural elucidation. Although this list of comparisons and contrasts could be further elaborated, suffice it to say that for trace amines (and metabolites) GC techniques offer the best practical possibilities, whereas for catecholamines, indoleamines and their metabolites HPLC-EC is the technique of choice. Comprehensive reviews of HPLC-EC theory and applications to neurochemistry may be found in Warsh et al. (1982), Krstulovic (1982), Hashimoto and Maruyama (1983), Kissinger (1983), and Mefford et al. (1983).

L. Outline of Research Programme, Including Objectives and Hypotheses

The research described in this thesis deals with three areas related to the use of analogs of bioactive amines to modify the brain levels and/or metabolism of these bioactive amines.

1. A major portion of the project was concerned with the testing of the following analogs of PEA as potential prodrugs of PEA: N-cyanoethyl-PEA (CEPEA), N-(3-chloropropyl)-PEA (CPPEA), N-propargyl-PEA (PGPEA) and N,N-dipropargyl-PEA (DPGPEA). These investigations included studies on rat brain, liver and blood concentrations of the prodrugs and PEA at various times after administration of each prodrug. To examine possible interactions of the prodrug and/or the PEA formed from it with putative neurotransmitter amines, brain levels of DA, NA, 5-HT and the metabolites DOPAC, HVA and 5-HIAA were also measured at the same time intervals after injection of the prodrugs. Since several of the prodrugs had structural similarities to known MAO inhibitors, MAO activity was also measured in the brain samples. In a smaller, preliminary

study, the N-cyanoethyl analog of tryptamine (CET) was also evaluated as a possible prodrug of T.

It was hypothesized that intraperitoneal injection of the N-cyanoethyl, N-(3-chloropropyl), N-propargyl and N,N-dipropargyl analogs of PEA and the N-cyanoethyl analog of T would lead to elevated, sustained brain levels of the parent amine (PEA or T) in question without elevating levels of other biogenic amines. Based on literature reports on metabolism of amphetamine analogs, it was hypothesized that CEPEA would be a more effective prodrug than CPPEA and that PGPEA and DPGPEA would also be effective prodrugs of PEA but might also cause inhibition of MAO.

2. Also investigated were N-alkylated and phenyl ring-substituted analogs of the MAO-inhibiting antidepressant TCP. This drug is structurally similar to amphetamine and PEA and, like those two amines, is rapidly cleared from the brain after intraperitoneal injection following attainment of high brain concentrations shortly after injection. Attempts were made to alter this clearance from brain by preparing the 4-fluoro analog (FTCP) in which para-hydroxylation on the ring would be blocked, and by preparing the N-alkylated analog N-propargyl-TCP which might act as a prodrug of TCP. The N-propargyl analog of PEA had proven to be an effective prodrug of PEA.

Pharmacological studies were carried out in which levels of TCP and FTCP or the N-alkylated prodrug were analyzed in brain, liver and blood of rats at various times after intraperitoneal injection of FTCP or the prodrug. Levels of FTCP (or TCP in the case of the prodrug) were compared to levels of TCP attained in rats which had been injected with an equimolar dose of TCP itself. To examine further the neurochemical actions of TCP, FTCP and the prodrug, investigations were made of levels of the neurotransmitter amines DA, NA and 5-HT in rat brain at the same time intervals.

Based on previous knowledge about hydroxylation of TCP and about propargyl analogs of amphetamine and PEA, the following hypotheses were made: (a) FTCP would attain higher and more prolonged levels in brain than TCP after intraperitoneal injection of an equimolar dose; (b) N-propargyl-TCP (PGTCP) would be an effective prodrug of TCP, resulting in sustained levels of TCP in brain, and (c) PGTCP would be a potent inhibitor of MAO.

In other experiments in this area, TCP, the prodrug and FTCP as well as several other analogs of TCP substituted on the phenyl ring or on the amine group were examined for their MAO-inhibiting properties _in vitro_ relative to TCP. It was hypothesized that N-substituted analogs of TCP would be weaker inhibitors of TCP than TCP itself while the ring-substituted analogs and 2-(naphthyl)-cyclopropylamine might be as potent as TCP.

3. In order to carry out most of the studies described above, it was necessary to develop a number of electron-capture gas chromatographic procedures for the analysis of both the bioactive amines and their respective analogs. This portion of the work involved the testing of two sensitive derivatizing reagents, namely PFBC and PFBSC, the selection of suitable separation conditions, and the confirmation of the structures of the final derivatives (using combined GC-MS) before applying the assay procedures routinely.

Based on preliminary work that had been done with amphetamine and a variety of secondary amines in the Neurochemical Research Unit, it was hypothesized that both reagents would be suitable derivatizing reagents for PEA, T and the drugs of interest in this project.

II. MATERIALS AND METHODS

A. Analytical Instrumentation and Apparatus

A.1 Gas chromatography

Gas chromatographic analyses were performed on Hewlett-Packard (HP) gas chromatographs each equipped with a 15 mCi ^{63}Ni linear ECD. Two different gas chromatographs were used:

1. A HP 5880A gas chromatograph equipped with a 25 m long fused silica capillary column (0.31 mm I.D., 0.5 μm film of 5% phenylmethylsilicone as stationary phase, Hewlett Packard Co., Palo Alto, U.S.A.) and interfaced with a HP 5880A (level 4) integrator to measure the peak heights.
2. A HP 5890 GC equipped with a 15 m long SE-54® fused silica capillary column (0.246 mm I.D., 0.25 μm film of 5% phenylmethylsilicone + 1% vinyl stationary phase, J. & W. Scientific, Inc., Rancho Cordova, CA, U.S.A.) and interfaced with a HP 3392A integrator.

Helium at a flow rate of 2 ml/min was employed as a carrier gas and 5% methane in argon at a flow rate of 35 ml/min was employed as a makeup gas at the detector on both gas chromatographs. The injector port temperature was 200°C, but the detector temperature and oven temperature programming were changed depending upon the analytical requirements and are described in the individual assay methods.

Measurements of trace amines and drugs in tissue samples were performed by determining the peak height ratios of the analytes to the internal standard (IS) in the sample and using them in linear regression equations of peak height ratios of known amounts of analytes to a fixed amount of IS (standard curve). A standard curve was included in each assay run and carried in parallel through the entire assay procedure. Suitable blanks were included in each assay run to correct for any possible interfering peaks in the chromatograms.

A.2 Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry was employed to determine the strucutures of the derivatives employed in the GC-ECD assays. These GC-MS analyses were performed using the same column that had been employed for GC-ECD analysis under identical temperature programming conditions. Two types of GC-MS systems have been employed:

1. A quadrupole VG-7070E mass spectrometer interfaced with a Vista-6000 Series Varian GC was employed in the EI mode. The operating conditions of the gas chromatograph were the same as those employed in the GC-ECD analysis; the operating conditions of the mass spectrometer system were as follows: ion source temperature, 250°C; interface temperature, 290°C; column pressure, 1.035 bar (15 p.s.i.); accelerating voltage, 6 kV; ionization voltage, 70 eV; scan speed, 0.5 sec/decade; dwell time, 0.5 sec. A PDP-11/73 computer was used to acquire the data.
2. A HP 5985A GC-MS system containing a quadrupole mass spectrometer was employed. The GC operating conditions were the same as those used in GC-ECD analysis. The mass spectrometer was operated under the following conditions: ion source temperature, 200°C; interface temperature, 275°C; column pressure, 0.69 bar (10 p.s.i.); accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 100

amu/sec; and dwell time, 200 msec. When CI was employed, methane was used as the reactant gas at a pressure of 3×10^{-4} Torr; the accelerating voltage was 2600 eV. The spectrometric data were handled by a HP 7620 disc drive, a HP 21MX series E computer with a HP 2648 graphics terminal and a HP 9676A printer.

A.3 High pressure liquid chromatography with electrochemical detection

Brain levels of catecholamines, 5-HT and the metabolites DOPAC, HVA and 5-HIAA were determined by reversed-phase HPLC-EC (RP-HPLC-EC).

The HPLC system was composed of a model 510 solvent delivery system, a model 710B sample injector system (WISP, Waters Associates) set to inject 20 μl of sample and a model 460 electrochemical detector (Waters Associates). The compounds of interest were electrochemically oxidized at a glassy carbon electrode set against a Ag/AgCl reference electrode at a voltage of 0.75 V. A HP 3392A integrator or Waters 740 data module linked to the HPLC system was employed to measure peak heights. A mobile phase composed of 55 mM NaH_2PO_4, 0.85 mM sodium octyl sulfate, 0.37 mM disodium EDTA and 9% acetonitrile was prepared and the pH was adjusted to 3.0 with phosphoric acid. The mobile phase was filtered through a type-HA filter (0.45 μm, Millipore) in a Millipore filtration system, degassed and pumped at a flow rate of 1 ml/min through a Econosphere-C_{18} (dimensions 4.6 mm x 250 mm, 5 μm particle size, Applied Science Labs, Avondale, PA, U.S.A.) column. A precolumn composed of same stationary phase as the analytical column was routinely employed. Brain samples were homogenized in a homogenization solution containing 0.1 mM perchloric acid, 0.1 mM sodium metabisulfite, 0.25 mM disodium EDTA and 50 ng/ml 3,4-dihydroxybenzylamine (DHBA) as an IS.

Using this system, NA, DA, 5-HT, DOPAC, HVA and 5-HIAA could be analysed in one run.

A.4 Liquid scintillation spectrometry

A Beckman LS 7500 (Beckman Co., Fullerton, CA, U.S.A.) liquid scintillation spectrometer interfaced with a Datamex Model 43 printer was employed. The LS 7500 is a microprocessor-controlled, two or three channel, 300-sample capacity, ambient temperature, soft beta-counting spectrometer. It has the features of automatic quench compensation (AQC), H-number quench monitor, and 10 library programs with unalterable memory. A scintillation cocktail containing 0.4% w/v of butyl-PBD in a mixture of Triton X-100 and toluene (33:66 v/v) was employed in the scintillation procedures.

A.5 Glassware

All glassware was soaked in a soluton of Sparkleen® (Fischer Scientific Co.), placed in ultrasonic cleaner (Mettler Electronics) and sonicated for 45 min in a solution of 2% Decan® (BDH chemicals), in water. The glassware was then washed in tap water and cleaned in a Miele dishwashing machine (Miele Electronics, F.R.G.) in a cold water-hot water-distilled water wash and rinse cycle. Finally the glassware was air dried at 250°-300°C for 1 hr in a mechanical convection oven (Model 28, Precision Scientific Group).

A.6 Balances

Tissue samples, drugs and chemicals were weighed on either a Metler AE160 or Sartorius 2003 MP1 electronic weighing balance with 0.1 mg sensitivity.

A.7 Tissue homogenizer

Tissue samples (brain, liver, lung, spleen, heart and kidney) for GC analysis were homogenized in ice-cold 0.1-0.4N perchloric acid (containing the appropriate antioxidants and IS) in a Tri-R Stir-R® (model S63C, Tri-R Instruments, Rockville, NY, U.S.A.) using a Teflon® pestle and a glass mortar (with a clearance of 0.1-0.15 mm). The rotor shaft has a maximum speed of 12000 rpm with a 10-speed setting. A speed setting of 8 was used routinely. In prepration for HPLC analysis, brain samples were homogenized in homogenizing solutions as described in Section II A.3. Blood samples were sonicated initially for 5 min in ice-cold 0.4N perchloric acid containing 10mg% disodium EDTA.

A.8 Centrifuges

All low-speed centrifugations (up to 1500 g) were performed on either a Sorvall GLC-2B or a Sorvall GLC-1 benchtop centrifuge (DuPont Instruments). High speed centrifugations were performed in a Damon IEC B-20 refrigerated centrifuge (Damon/IEC, Needham Hts., MA, U.S.A.) at 12000 g for 10-15 min at 0-4°C. For small-volume centrifugations, a MSE Micro-Centaur benchtop centrifuge was employed at high speed.

A.9 Shaker-mixer

A IKA-VIBRAX-VXR® (Janke & Kunkel GmbH, Sweden) tube shaker was employed for extraction purposes. This shaker-mixer has the capacity to hold 36 tubes. For mixing and vortexing individual tubes, a benchtop Thermolyne Maxi Mix 1® (Sybron Instruments, Dubuque, IA, U.S.A.) vortexer was employed.

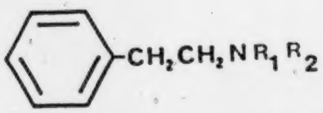
B. Chemicals

The following is a list of the names of the chemicals employed in this study and their respective suppliers or manufacturers.

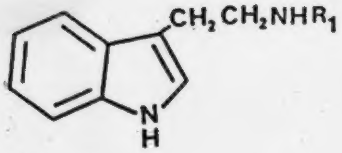
Chemicals	Suppliers or Manufacturers
2-Phenylethylamine (PEA) HCl, tryptamine (T) HCl, benzylamine (BZA) HCl, *p*-chlorophenylethylamine* (CPEA), (±)-tranylcypromine (TCP) HCl, (-)-noradrenaline (NA) HCl, dopamine (DA) HCl, 5-hydroxytryptamine (5-HT) creatinine sulfate, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA), 3,4-dihydroxybenzylamine (DHBA) HCl, butyl-PBD, [2-(4^{1}-tertbutylphenyl)-5-(4^{11}-biphenyl)-1,2,3,-oxadiazole], di-(2-ethylhexyl)-phosphate (DEHPA), pargyline (PAR) HCl	Sigma Chemical Co. (St. Louis, MO, U.S.A.)
Pentafluorobenzoyl chloride (PFBC), pentafluorobenzenesulfonyl chloride (PFBSC), octyl sodium sulfate (SOS)	Aldrich Chemical Co. (Milwaukee, WI, U.S.A.)
Pentafluoropropionic anhydride (PFPA), trifluoroacetic anhydride (TFAA),	Pierce Chemical Co. (Rockville, IL, U.S.A.)
Triton X-100	Terochem Laboratories (Edmonton, Alta., Canada)
Chloroform (ACS), toluene (glass-distilled), ethyl acetate (glass-distilled), acetonitrile (HPLC grade), methanol (HPLC grade)	Caledon Labs (Georgetown, Ont., Canada)
Ammonium hydroxide, sodium carbonate, potassium bicarbonate, potassium carbonate, perchloric acid (60%), hydrochloric acid	Fisher Scientific (Fairlawn, NJ, U.S.A.)
Hydroxytryptamine binoxalate, 5-[$2^{14}C$] (sp. activity 55 mCi/mmol); phenylethylamine HCl, 2-[ethyl-1-^{14}C] (sp. activity 50.2 mCi/mmol]	New England Nuclear (NEN) (Boston, MA, U.S.A.)
Sodium phosphate dibasic anhydrous, sodium acid phosphate	Amacham (Seattle, WA, U.S.A.)
Phenelzine (PLZ) sulfate	ICN Pharmaceuticals (Plainview, NY, U.S.A.)

Chemicals	Suppliers
Isopentane (2-methylbutane)	Eastman Kodak (Rochester, NY, U.S.A.)
2,6-Dichlorophenoxypropylamine	May & Baker Ltd. (London, U.K.)
Sodium metabisulfite	J.T. Baker & Co. (Philipsburg, NJ, U.S.A.)
N-(2-Cyanoethyl)-2-phenylethylamine (CEPEA) HCl, N-(3-chloropropyl)-2-phenylethylamine (CPPEA) HCl, N-propargyl-2-phenylethylamine (PGPEA) HCl, N,N-propargyl-2-phenylethylamine (DPGPEA) HCl, N-methyl,N-propargyl-2-phenylethylamine (MPGPEA) HCl, N-(2-cyanoethyl) tryptamine (CET) HCl, *p*-fluorotranylcypromine (FTCP) HCl, *p*-methoxytranylcypromine (MTCP) HCl, 3,4-methylenedioxytranylcypromine (MDTCP) HCl, 3-trifluoromethyltranylcypromine (TTCP) HCl, N-acetyltranylcypromine (ATCP), N-propargyltranylcypromine (PGTCP) HCl, N-ethoxycarbonyltranylcypromine (ECTCP), N-isobutoxycarbonyltranylcypromine (ICTCP), 2-naphthylcyclopropylamine (NCP) HCl, N-(2-cyanoethyl)tranylcypromine (CETCP) HCl	Drs. T. W. Hall, R. G. Micetich and R. T. Coutts Faculty of Pharmacy and Pharmaceutical Sciences University of Alberta (Edmonton, Alta., Canada)

*converted into HCl salt by Dr. T.W. Hall

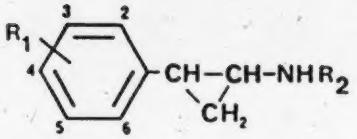

$CH_2CH_2NR_1R_2$

A. $R_1=R_2=H$	PEA
B. $R_1=H$, $R_2=CH_2CH_2CN$	CEPEA
C. $R_1=H$, $R_2=CH_2CH_2CH_2Cl$	CPPEA
D. $R_1=H$, $R_2=CH_2C\equiv CH$	PGPEA
E. $R_1=R_2=CH_2C\equiv CH$	DPGPEA
F. $R_1=CH_3$, $R_2=CH_2C\equiv CH$	MPGPEA

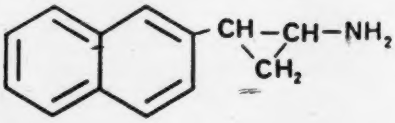

$CH_2CH_2NHR_1$
N
H

G. $R=H$	T
H. $R_1=CH_2CH_2CN$	CET

Fig. 11. Structures of trace amines and their prodrugs.


R1
3
2
4
5
6
CH–CH-NHR2
CH2

A.	$R_1 = H, R_2 = H$	TCP
B.	$R_1 = H, R_2 = COCH_3$	ATCP
C.	$R_1 = 4\text{-}OH, R_2 = H$	HTCP
D.	$R_1 = 4\text{-}F, R_2 = H$	FTCP
E.	$R_1 = 3\text{-}CF_3, R_2 = H$	TTCP
F.	$R_1 = 4\text{-}OCH_3, R_2 = H$	MTCP
G.	$R_1 = H, R_2 = CH_2C{\equiv}CH$	PGTCP
H.	$R_1 = 3,4\text{-}(O\text{-}CH_2\text{-}O), R_2 = H$	MDTCP
I.	$R_1 = H, R_2 = COOCH_2CH_3$	ECTCP
J.	$R_1 = H, R_2 = COOCH(CH_3)_2$	ICTCP
K.	$R_1 = H, R_2 = CH_2CH_2CN$	CETCP


CH–CH–NH2
CH2

L. NCP

Fig. 12. Structures of TCP analogs.

B.1 Synthesis of prodrugs of trace amines and analogs of TCP

Detailed synthetic methods and spectroscopic data on prodrugs of PEA and *T* are reported elsewhere (Coutts *et al.*, 1986c). A brief description of the synthesis of structural analogs of TCP is given below.

Tranylcypromine and its analogs were synthesized from their corresponding cinnamic acids by Trost's method as modified by Kaiser *et al.* (1965). The appropriate cinnamic acid was obtained either from Aldrich Chemical Co. or synthesized from the corresponding benzaldehydes and malonic acid by the procedure described by Wiley and Smith (1963). The cinnamic acid was converted to its acid chloride by reaction with thionyl chloride in refluxing benzene. The resultant acid chloride was converted to its *tert*-butyl ester by reacting with dry *tert*-butyl alcohol and pyridine in methylene chloride as solvent. The *tert*-butyl cinnamate ester was then converted into its cyclopropyl ester by the reacting with the ylide generated from trimethylsulfoxinium iodide and sodium hydride in dry dimethyl sulfoxide. The cyclopropyl *tert*-butyl ester was hydrolysed to its corresponding cyclopropane carboxylic acid in the presence of trifluoroacetic acid and the acid was converted to a acid chloride by reacting with thionyl chloride in refluxing benzene. The acid chloride was converted to its acylazide by reacting with sodium azide in the presence of *tetra*-butylammonium bromide (Pfister and Wyman, 1983). The acylazide was converted into a *tert*-butylcarbamate by a Curtis rearrangement upon the addition of *tert*-butyl alcohol. The carbamate was purified by recrystallization and hydrolysed by trifluoroacetic acid to form the corresponding TCP analog. The free base was converted to its HCl salt and purified by recrystallization.

N-Propargyl-TCP (PGTCP) was prepared by reacting 1 mole of TCP in methylene chloride with 1 mole of propargyl bromide (Popov *et al.*, 1967). The resultant PGTCP was isolated from the reaction products by column chromatography over silica gel.

All structures were confirmed by ^{13}C nuclear magnetic resonance, infrared spectroscopy and mass spectrometric analysis.

C. Animals

Male Sprague-Dawley rats (175-230 g) were obtained from Bio Science Animal Services, Ellerslie, Alberta, Canada. The animals were housed in plastic cages on cedar chip bedding in a temperature-controlled room (21±1°C), with an alternate 12 hr light and dark schedule. Food and water were provided *ad libitum*. Animals were fed with Lab-Blox Feed (from Wayne Food Division, Continental Grain Co., Chicago, U.S.A.), composed of 4.0% crude fat (min.), 4.5% crude fibre (max.), and 24% crude protein (min.).

C.1 Administration of drugs

Groups of rats were administered intraperitoneally with drugs (in isotonic saline) at a dose of 0.1 mmol/kg and were sacrificed at predetermined time intervals (5, 15, 30, 60, 90, 120, 180 and 240 min in the case of prodrugs of trace amines or trace amines (PEA, T) themselves, and at 0.5, 1.5, 3.0, 6.0, 12.0, 16.0 and 24.0 hr in the case of TCP, FTCP and PGTCP) by cervical dislocation followed by immediate decapitation. Blood samples from the neck region of the decapitated animals were collected in plastic vials into which 100 µl of saturated disodium EDTA solution had been previously added; the contents were thoroughly mixed and frozen solid by immersing in isopentane on solid carbon dioxide. Brains (meninges and pineal gland removed) and liver samples were rapidly dissected out and frozen solid in isopentane on solid carbon dioxide. All the tissue samples were stored at -60°C until the time of analysis. Analysis of these samples was completed within 2-3 weeks of tissue collection.

D. Analysis of Trace Amines, Prodrugs of Trace Amines and MAO Inhibitors

D.1 Analysis of PEA and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) in rat brain, blood and liver by extractive pentafluorobenzoylation

Analysis of PEA and CEPEA in brain, blood and liver was carried out by a modification of the method of Nazarali *et al.* (1986a). Partially thawed tissues were weighed and homogenized in 5 volumes of ice cold 0.4 N perchloric acid. The homogenates were centrifuged at 10,000 g for 15 min at 0-4°C. Aliquots (3 ml) of homogenates were transferred to a set of test tubes and 2000 ng of the IS (2,6-dichlorophenoxypropylamine) was added to all tubes. In the case of blood, the samples were allowed to thaw completely and 1 g portions were weighed in polyethylene tubes into which 2 ml of 0.4N perchloric acid was added; the tubes were sonicated for 5 min, centrifuged at 1000 g for 3 min, and the entire clear supernatants were used. Simultaneously, a set of standards containing varying amounts of PEA and CEPEA and 2000 ng of the IS was also included in each assay to be carried through the entire procedure. Excess acid was neutralized by the addition of solid $KHCO_3$ and the precipitate of potassium perchlorate was removed by centrifugation (1000 g, 5 min). The supernatants were adjusted to pH 7.8 and extracted with 3 volumes of chloroform containing DEHPA (2.5% v/v) by shaking vigorously for 3 min. Following a brief centrifugation, the aqueous layers were aspirated off and the organic layers were transferred to clean tubes and extracted for 2 min with equal volumes of 0.5 N HCl. After a brief centrifugation, the aqueous layers were removed and transferred to another set of clean tubes. The PEA, CEPEA and IS were converted to their N-pentafluorobenzoyl (PFB) derivatives by basifying the aqueous layer with solid K_2CO_3 and shaking vigorously for 15 min with 3 ml of a mixture of toluene:acetonitrile:PFBC (9:1:0.01 v:v:v). After centrifugation, the organic layers were transferred to another set of tubes and taken to dryness under a stream of nitrogen. The residues were dissolved in 300 µl of toluene and briefly washed with 500 µl of 1.0 N NH_4OH. After a brief centrifugation, the toluene layers were transferred to microfuge tubes and a 1 µl aliquot from each tube was injected onto the gas chromatograph.

The HP 5880A gas chromatograph system (Section II.A.1) was used with a detector temperature of 300°C. The oven temperature was programmed to increase from an initial temperature of 105°C (held for 0.5 min) at a rate of 25°C/min to 280°C (held for 10 min).

D.2 Analysis of PEA, N-(3-chloropropyl)-2-phenylethylamine (CPPEA) and N-propargyl-2-phenylethylamine (PGPEA) in rat brain, blood and liver by extractive pentafluorobenzoylation

Levels of PEA, CPPEA and PEPEA in rat brain, blood and liver were determined by a slight modification of the extractive pentafluorobenzoylation method described in Section II.D.1. The extraction step using DEHPA in chloroform was omitted and the rest of the procedure was the same.

The HP 5890 GC system (Section II.A.1) was employed with a detector temperature of 345°. The oven temperature was programmed to increase from 105° (held for 0.5 min) at a rate of 25°/min to 300° (held for 10 min).

D.3 Analysis of PLZ in rat brain and patient urine samples by aqueous pentafluorobenzoylation

The pentafluorobenzoylation procedures described above were modified to provide an assay for PLZ, a MAO inhibitor which had proved difficult to assay in the past (Rao et al., 1986c).

Partially thawed brain samples were weighed, cut into small pieces and homogenized in 5 volumes of ice-cold 0.4N perchloric acid containing 10 mg% sodium EDTA. The homogenates were centrifuged at 12,000 g for 15 min at 0-4°. Aliquots (3 ml) of supernatants were transferred to a set of tubes and 500 ng of the IS (CPEA) was added to each tube. A set of standard tubes each containing varying amounts of authentic PLZ and 500 ng of CPEA in 3.0 ml of 0.4N perchloric acid was also included in each assay and carried through the entire procedure. For the analysis of PLZ in urine samples from human subjects, 3 ml urine samples with added IS were included in the assay. Urine samples were acidified with 100 µl of 0.4N $HClO_4$ with 10 mg% EDTA and centrifuged at 1000 g for 5 min to remove suspended matter. The clear supernatants were transferred to another set of tubes. Excess acid was neutralized by the addition of solid $KHCO_3$, and the resultant precipitate was removed by centrifugation (1000 g, 3 min). The clear supernatants were transferred to another set of tubes, 1/10 the volume of 0.1 M phosphate buffer (pH 7.8) was added and the solutions were extracted by shaking for 5 min with 3 ml of the liquid ion-pairing agent DEHPA (2.5% v/v in chloroform). Following a brief centrifugation, the upper aqueous layers were aspirated off and the organic layers were each back-extracted with 2.5 ml of 0.5N HCl. The samples were centrifuged briefly to separate the phases and the aqueous layers were transferred to another set of tubes and basified by the addition of solid $KHCO_3$ (sufficient that a small excess remained on the bottom of the tubes after shaking). A solution (3 ml) of toluene: acetonitrile:PFBC (9:1:.015 v:v:v) was added to each tube, and the tubes were shaken vigorously for 10 min. A further 2 µl of PFBC in 20 µl of toluene was added to each, shaking was continued for 10 min, and the samples were centrifuged. The organic layers were transferred to another set of tubes and taken to dryness under a stream of nitrogen. The residues were each taken up in 300 µl of toluene and washed rapidly with 500 µl of 1.0N NH_4OH. The toluene layers were transferred to microfuge tubes (400 µl size) and a 1 µl aliquot of each was used for GC-ECD analysis in each case.

The Hewlett Packard (HP) 5890 GC system was employed with a detector temperature of 345°. The oven temperature was programmed to increase from 105° (held for 0.5 min) at a rate of 25°/min to 300° (held for 10 min).

Quantitation was carried out by determining the peak height ratios of PLZ to IS in each sample and comparing the ratios to values on the calibration curve obtained from the standards which were carried through in parallel with each assay run. The calibration curve consisted of plots of quantities of authentic PLZ standards versus the corresponding PLZ/IS peak height ratios.

D.4 Analysis of PEA in rat tissues and body fluids using aqueous pentafluorobenzenesulfonylation

A method to quantitate PEA levels in rat brain, blood, liver, heart, spleen, lung, kidney and in human urine samples has been developed (Baker et al., 1986a), employing PFBSC as a novel derivatizing agent.

Analysis of PEA in tissues of the rat. The method employed was essentially the same as that described under Section II.D.2 except that a 3 ml mixture of toluene:acetonitrile:PFBSC was employed instead of a solvent mixture containing PFBC. The reaction time was limited to 2 min.

Analysis of PEA in human urine samples. After thawing, portions (4 ml) of urine were transferred to clean tubes. The IS CPEA (500 ng) was added to each tube and the samples were basified by addition of solid $KHCO_3$ and centrifuged briefly at 1000 g for 3 min. The clear supernatants were transferred to another set of tubes, 400 µl of sodium phosphate buffer (pH 7.8) was added, and the urines were extracted by shaking with 4 ml of chloroform containing the liquid ion-pairing agent DEHPA (2.5% v/v). Following a brief centrifugation, the top aqueous layers were aspirated off and the chloroform layers were vigorously shaken with 2.5 ml of 0.5N HCl for 5 min. The aqueous acid layers were transferred to another set of tubes following a brief centrifugation. The subsequent derivatization procedure was the same as described for the analysis of PEA in the tissues of rat except that 4 ml of the derivatizing mixture was used.

The HP 5890 GC system was employed with a detector temperature of 300°C. A two-level oven temperature programme was used: the initial temperature of 105° was maintained for 0.5 min, then the oven was heated at 30°/min to 255°, at which temperature it was held for 1 min; this was followed by an increase (at 25°/min) to 280°, and the final temperature was maintained for 10 min.

D.5 Analysis of TCP and para-fluorotranylcypromine (FTCP) by aqueous pentafluorobenzenesulfonylation

A rapid and sensitive method was developed for the analysis of TCP and FTCP in rat tissues and biological fluids employing reaction with PFBSC under aqueous conditions (Rao *et al.*, 1986a; Coutts *et al.*, 1986a). The method involved the same procedures described under Section II.D.4 for analysis of PEA in rat tissues.

The above procedure works well for the tissue and blood samples described. However, if further "cleanup" is required (e.g. urine samples), an additional purification step may he added prior to derivatization. Briefly this involves adjustment of the pH of the sample to 7.8 and shaking for 5 min with 4 ml of chloroform containing the liquid ion-pairing compound DEHPA (2.5% v/v). Following brief centrifugation, the upper aqueous layers are aspirated off, and the TCP is back-extracted from the chloroform layer by shaking for 5 min with 0.5 M HCl (3 ml). The acid layers are transferred to another set of tubes, and the TCP they contain is derivatized using the procedure described above.

Gas chromatography. The HP 5890A GC system was used with a temperature of 300°. The oven temperature was programmed to increase from 105° (maintained for 0.5 min) at a rate of 30°/min to 255° (maintained for 1 min) and then to increase at a rate of 25°/min to 280° (maintained for 10 min).

D.6 Analysis of T in rat brain, blood and liver samples by aqueous pentafluorobenzenesulfonylation

The rat brain and liver levels of T were determined by a modification to earlier reported methods (Baker et al., 1986a; Coutts et al., 1986a; Rao et al., 1986a) which employ GC-ECD for detection and quantitation of the amine after aqueous derivatization with PFBSC (Section II.D.4 and D.5). 5-Methytryptamine (5-MT) (1000 ng) was employed as IS and the reaction time was extended to 5 min instead of the 2 min described in Sections II.D.4 and D.5.

D.7 Simultaneous analysis of PEA, TCP, FTCP and T by aqueous pentafluorobenzenesulfonylation

Pentafluorobenzenesulfonyl chloride has been employed for the analysis of PEA (Baker et al., 1986a), TCP (Rao et al., 1986a), FTCP (Coutts et al., 1986a) and T individually. A single method for the simultaneous analysis of PEA, T and TCP (or FTCP) can be achieved by extractively derivatizing alkaline tissue extracts for 5 min with a 3 ml mixture of toluene:acetonitrile:PFBSC (9:1:0.01:v/v/v) as described above. The amine CPEA is used as an IS. Subsequent methods and GC conditions are same as those described under Sections II.D.4, II.D.5 and II.D.6.

D.8 Simultaneous analysis of T and N-(2-cyanoethyl)-tryptamine (CET) in rat brain, blood and liver samples by aqueous pentafluorobenzenesulfonylation

Simultaneous determination of T and CET, a compound designed as a prodrug of T, can be performed using an extension of the technique described previously (Section II.D.6) for analysis of T.

The assay procedure involved essentially the same steps as described in Section II.D.6 except that the reaction time was extended to

10 min for complete derivatization of T and CET. Subsequent methods and GC conditions are the same as those described under Sections II.D.6 and II.D.7.

D.9 Simultaneous analysis of (PEA), TCP, N-propargyltranylcypromine (PGTCP) and T by aqueous pentafluorobenzenesulfonylation

N-Propargyl-TCP was examined as a prodrug of the MAO inhibitor TCP. The aqueous pentafluorobenzenesulfonylation technique described above was modified for the simultaneous analysis of PEA, T, TCP and PGTCP. The derivatization method was identical to the one described in Section II.D.8 except that 1000 ng of BZA instead of 5-MT was employed as IS and the reaction time was extended to 15 min.

E. Estimation of MAO Inhibition In Vitro and In Vivo

A modification of the method of Wurtman and Axelrod (1963) was employed to determine inhibition of MAO *in vitro* and *in vivo* by various test compounds. For *in vitro* assays rat whole brains were employed as the source of MAO. Brains were homogenized in 5 volumes of ice-cold isotonic KCl solution in a homogenizer and 200 μl portions were diluted to 1000 μl with isotonic KCl; aliquots (25 μl) of this diluted homogenate were employed in all *in vitro* studies. Test compounds available as HCl salts were directly dissolved in isotonic KCl, whereas the compounds available as free bases or insoluble in isotonic KCl were dissolved in small volumes of dimethylsulfoxide (DMSO) and diluted with isotonic KCl (appropriate blanks were included in the assay). To tubes kept on ice 250 μl phosphate buffer (pH 7.4) and 25 μl of tissue homogenate (25 μl of isotonic KCl in blanks) were added. Dilutions of test compounds were added to give the desired inhibitor concentration (equal volumes of appropriate solvents were added to blanks and controls). The tubes were preincubated for 10 min at 37°C after they were kept on ice for 5 min. Aliquots (25 1) of solutions of ^{14}C-5-HT (selective substrate for MAO-A) or ^{14}C-PEA (selective substrate for MAO-B) diluted suitably with respective unlabelled compounds were added to each tube to give a final concentration of 35 M of substrate in each. The tubes were incubated for 20 min at 37°C. After the incubation period, tubes were allowed to cool on ice and the enzyme reaction was stopped by the addition of 200 1 of 2M HCl. The acid metabolites formed were extracted into 6 ml of toluene by vigorous shaking for 3 min. The tubes were centrifuged at 1000 g for 5 min and kept at -70°C for 20 min or until the bottom aqueous layers were frozen solid. The top toluene layers were transferred to plastic vials containing 9 ml of scintillation cocktail. The vials were capped tightly and vortexed to mix the contents thoroughly. The radioactivity in each sample was measured by liquid scintillation spectrometry in a LS 7500 Beckman Spectrometer. The amount of radioactivity in blank tubes was subtracted from all samples and controls were averaged. The radioactivity in the sample was divided by that in controls and the value multiplied by 100 to give % activity. Percent inhibition was determined by subtracting % activities from 100.

The IC_{50} values (minimum inhibitor concentrations required to cause 50% inhibition of MAO) were determined by estimating MAO inhibitions at 5 different concentrations of the inhibitor, with each concentration replicated 6 times. Percentage inhibition data were plotted against logarithmic inhibitor concentrations, from which IC_{50} values were determined.

In the case of in vivo inhibition assays, 25 μl aliquots of isotonic KCl homogenates of brain or liver samples of rats treated with appropriate inhibitors were used instead of inhibitor solutions. To the control tubes, homogenates of saline-treated rat tissues were added and the preincubation step was omitted. In cases where MAO activity was measured in the same brain samples used for analysis of PEA, TCP, T and their respective prodrugs, the brain samples were homogenized in ice-cold isotonic KCl solution and an aliquot (200 μl) was removed and diluted with KCl solution for the MAO assay; to the remaining homogenate was added sufficient perchloric acid and EDTA to give the concentrations required in the assay for the amines and prodrugs.

Strictly speaking, these assays actually represent ex vivo assays since the tissue involved is removed from the rat before assaying for MAO activity. However, they will be referred to hereafter in this thesis as in vivo assays to indicate that the drug was injected in vivo and to differentiate them from in vitro assays in which the drugs are added to the tissue after removal of the tissue from the animal.

F. Determination of Rat Brain Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA

Brain levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA were analysed by HPLC-EC. Brain halves (the other halves had been used for GC analysis and measurement of MAO activity) were homogenized in 5 volumes of a solution containing perchloric acid (0.1 M), sodium metabisulfite (0.1 mM), disodium EDTA (0.25 mM), and the IS DHBA (50 ng/ml). The homogenates were centrifuged at 12000 g at 4°C for 15 min and a 200 μl aliquot of each of the clear supernatants was diluted to 600 or 1000 μl, depending upon the concentration of the neurotransmitters and metabolites in the sample. An aliquot (20 μl) of each was injected into the HPLC system described under Section II.A.3.1.

G. Statistical Analyses

Data were analysed by either one-way or two-way analysis of variance followed by independent t-tests in the case of single pair comparisons (P values indicated in the text) and by the Newman-Keuls test in the case of multiple mean comparisons. All the probabilites are two-tailed. A probability value $P<0.05$ (i.e., $\alpha=0.05$) was used to establish statistical significance. Statistical analyses were performed on a HP83 microcomputer using a statistical software package (HP) or on a Commodore VIC20 computer using statistical programs written by Dr. A. J. Greenshaw, Department of Psychiatry, University of Alberta.

H. Pharmacokinetic Calculations

Concentrations of analytes were plotted on semilog paper to determine the pharmacokinetic parameters. The technique of feathering (or stripping or the method of residuals) was applied to determine kinetic constants of distribution and elimination (Gibaldi and Perrier, 1982). Essentially, this involved extrapolation of the linear terminal portion of the curve towards the x-axis and subtraction of extrapolated concentrations from the observed concentrations. These differences or residuals were plotted on a semilogarithmic scale to give a straight line with a slope of $-K/2.303$. From these slopes the apparent first-order fast and slow elimination rate constants α and β could be characterized. These calculations were based on the assumption that the kinetics exhibit a two-compartment model. Sometimes it is necessary to apply the method of residuals more than once to characterize the fast elimination phase, and this indicates that the kinetics involve fairly complex transfer processes.

Estimations of areas under concentration-time curves (AUCs) for each tissue (brain, blood and liver) were required for pharmacokinetic analysis and were made using the trapezoidal rule (Gibaldi and Perrier, 1982).

III. RESULTS

A. Analysis of Trace Amines, Prodrugs of Trace Amines, TCP, TCP Analogs and PLZ

A.1 Simultaneous analysis of PEA and CEPEA by extractive pentafluorobenzoylation and GC-ECD

Using the conditions described in Section II.D.1, the retention times of derivatized PEA, CEPEA, and internal standard were 7.9, 9.5 and 12.6 min respectively. A typical GC trace is presented in Fig. 15. The structures were confirmed by GC-MS (Figs. 13 and 14) using the system described under Section II A.2.1. The standard curves were linear in the concentration range of 1-2000 ng/3 ml with a correlation coefficient $r > 0.99$ and the PFB derivatives were stable up to a month at -20°C.

A.2 Simultaneous analysis of PEA and CPPEA or PGPEA by extractive pentafluorobenzoylation and GC-ECD

The extraction and derivatization procedures were described in Section II.D.2. Derivatized PEA, PGPEA, internal standard and CPPEA displayed retention times of 5.2, 5.4, 6.1 and 6.4 min respectively, and typical GC traces of brain samples are presented in Figs. 16 and 17. The standard curves were linear in the range of 1-1000 ng/3 ml of PEA and 10-3000 ng/ml of PGPEA and CPPEA, with correlation coefficients $r>0.99$. The derivatives were stable up to a month at -20°C. Structures were confirmed by employing the GC-MS system described under Section II.A.2.2, and the proposed mass spectral fragmentations are presented in Figs. 18 and 19.

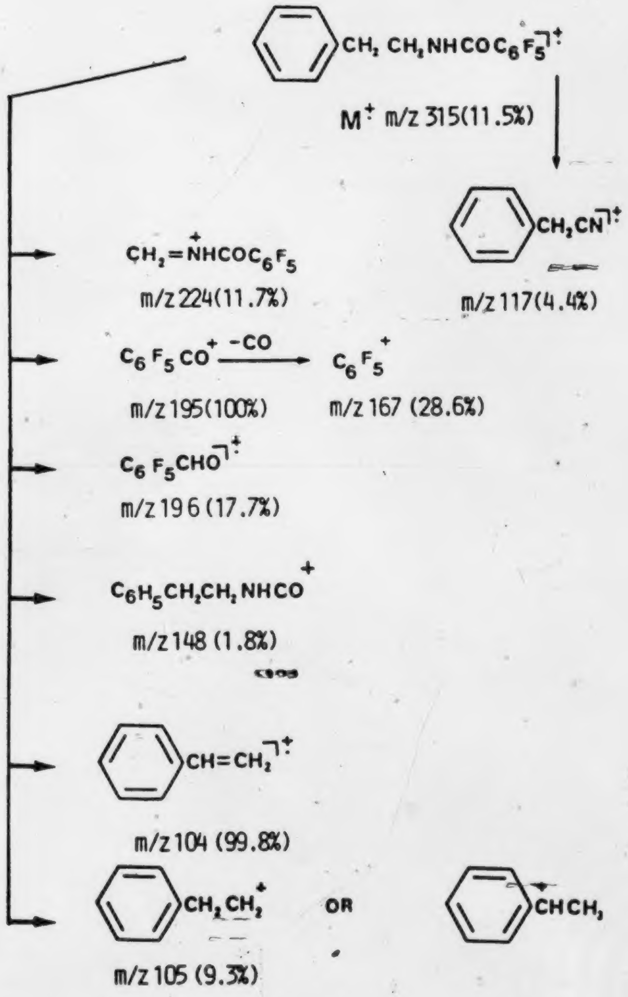

$CH_2CH_2NHCOC_6F_5$
$M^{+\cdot}$ m/z 315(11.5%)
CH_2CN
m/z 117(4.4%)
$CH_2=\overset{+}{N}HCOC_6F_5$
m/z 224(11.7%)
$C_6F_5CO^+$ -CO $C_6F_5^+$
m/z 195(100%)
m/z 167 (28.6%)
C_6F_5CHO
m/z 196 (17.7%)
$C_6H_5CH_2CH_2NHCO^+$
m/z 148 (1.8%)
$CH=CH_2$
m/z 104 (99.8%)
$CH_2CH_2^+$
OR
$CHCH_3$
m/z 105 (9.3%)

Fig. 13. Probable mass spectral fragmentation of the PFB derivative of PEA. Numbers in parenthesis indicate relative abundance of each fragment.

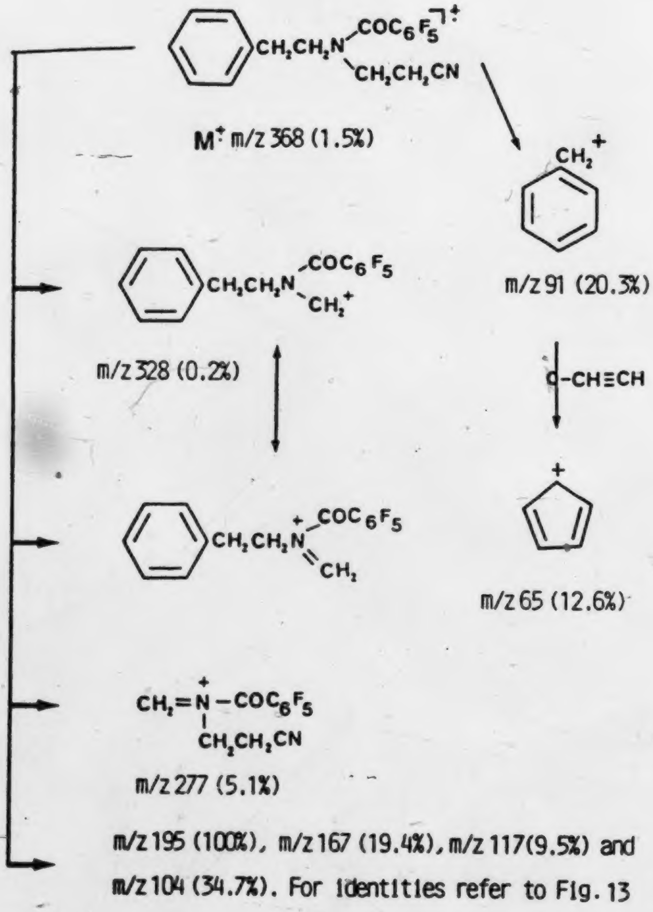

M⁺˙ m/z 368 (1.5%)
m/z 91 (20.3%)
-CH≡CH
m/z 328 (0.2%)
m/z 65 (12.6%)
m/z 277 (5.1%)
m/z 195 (100%), m/z 167 (19.4%), m/z 117 (9.5%) and m/z 104 (34.7%). For identities refer to Fig. 13

Fig. 14. Probable mass spectral fragmentation of the PFB derivative of CEPEA.

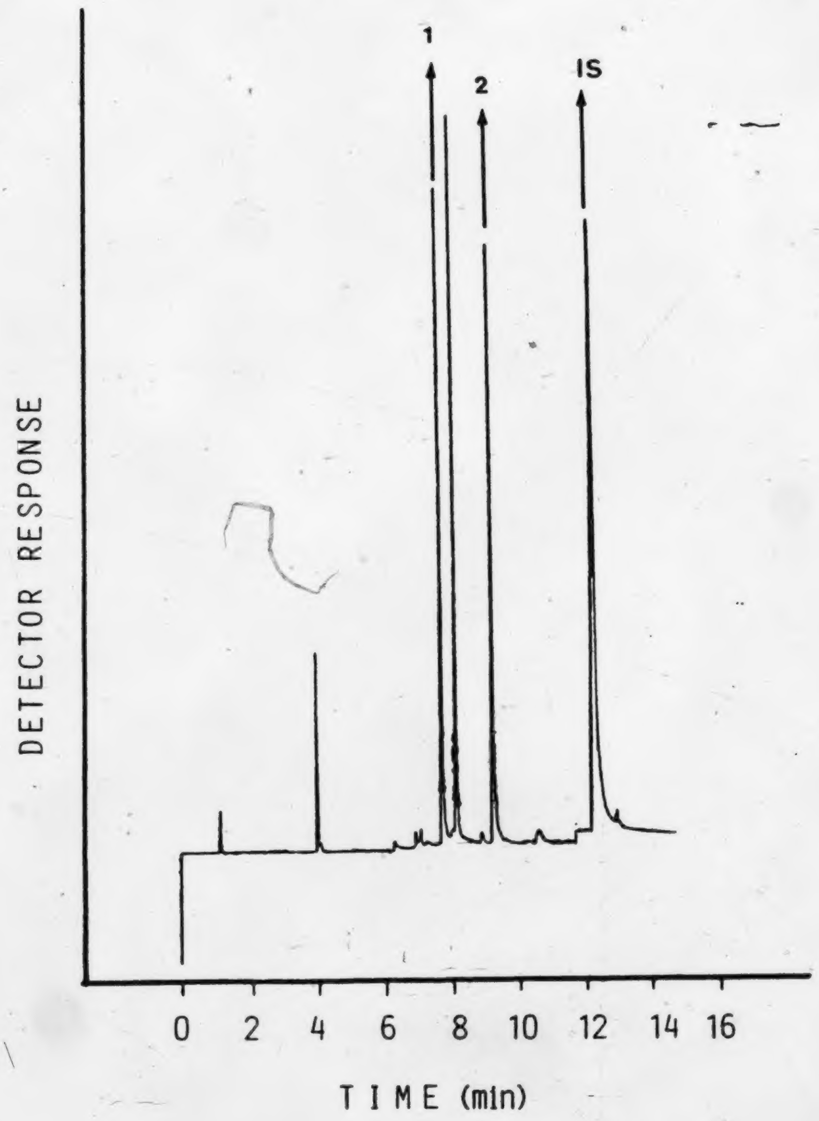


Fig. 15. A GC trace of an extract of CEPEA-treated (0.1 mmol/kg, i.p., 60 min) rat brain sample following derivatization with PFBC. The trace was recorded at an attenuation value of 2↑10 from 6 to 14 min. IS=2,6-dichlorophenoxypropylamine, 1=derivatized PEA, 2=derivatized CEPEA.

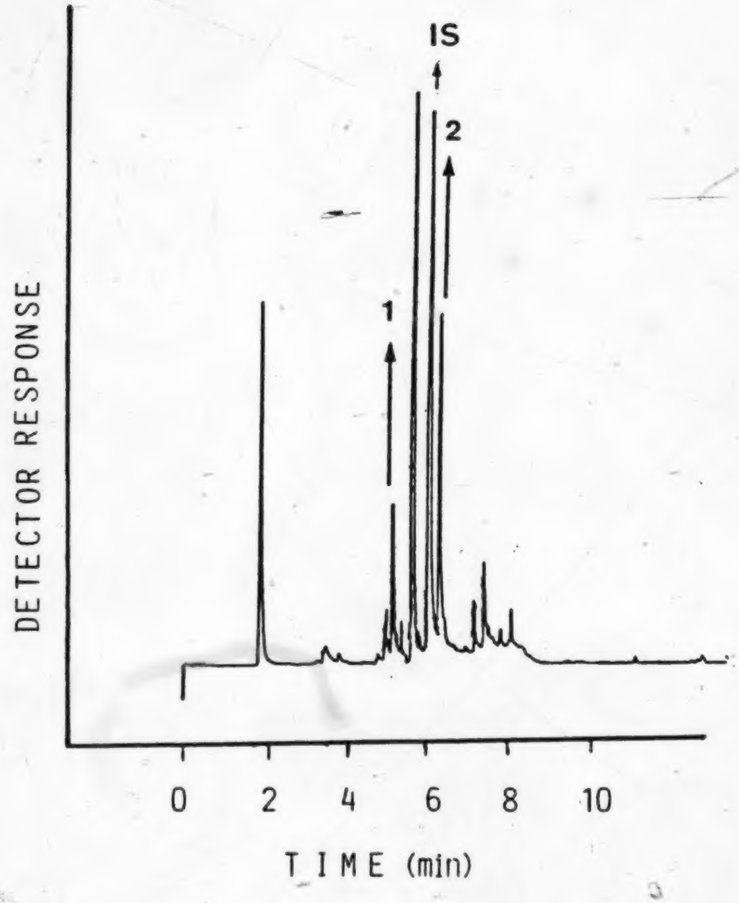


Fig. 16. A GC trace of an extract of CPPEA-treated (0.1 mmol/kg, i.p., 60 min) rat brain sample following derivatization with PFBC. The trace was recorded at an attenuation of 2↑6 from 4 to 8.3 min. 1=derivatived PEA, 2=derivatized CPPEA, IS=derivatized CPEA.

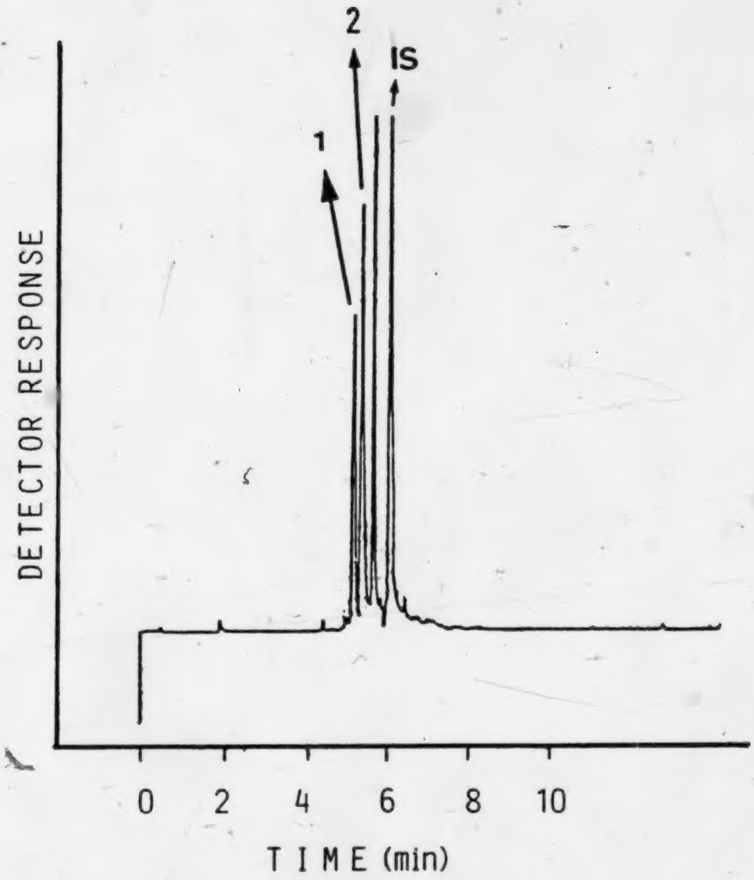
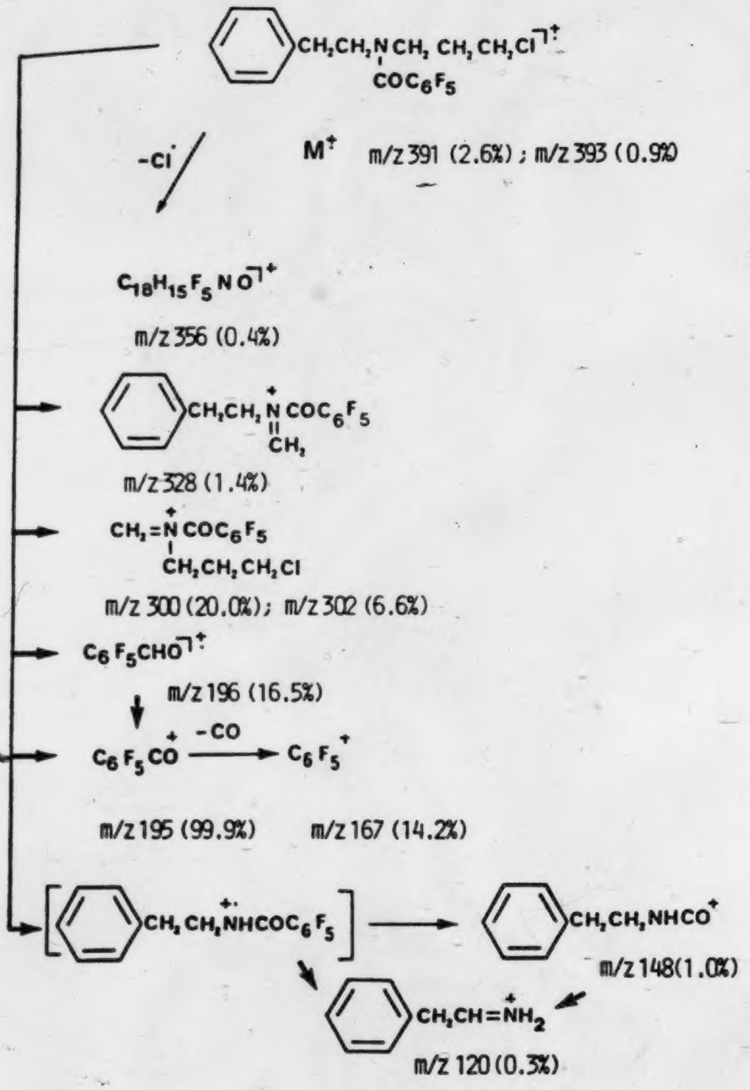
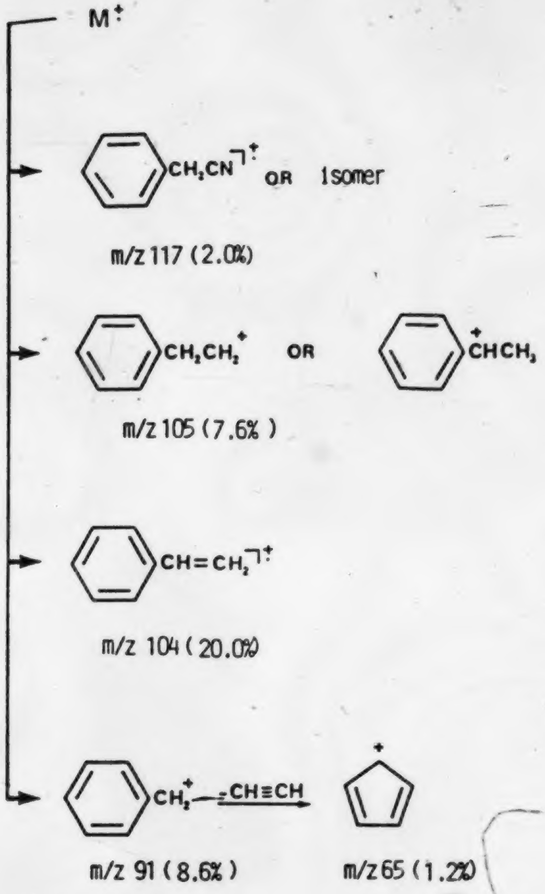


Fig. 17. A GC trace of an extract of PGPEA-treated (0.1 mmol/kg, i.p., 60 min) rat brain sample following derivatization with PFBC. The trace was recorded at an attenuation of 2↑7 from 4.1 to 7.5 min. 1=derivatived PEA, 2=derivatized PGPEA, IS=derivatized CPEA.


CH₂CH₂NCH₂CH₂CH₂Cl ⁺·
COC₆F₅
M⁺· m/z 391 (2.6%) ; m/z 393 (0.9%)
-Cl·
C₁₈H₁₅F₅NO ⁺·
m/z 356 (0.4%)
CH₂CH₂N⁺COC₆F₅
CH₂
m/z 328 (1.4%)
CH₂=N⁺COC₆F₅
CH₂CH₂CH₂Cl
m/z 300 (20.0%); m/z 302 (6.6%)
C₆F₅CHO ⁺·
m/z 196 (16.5%)
C₆F₅CO⁺
-CO
C₆F₅⁺
m/z 195 (99.9%)
m/z 167 (14.2%)
CH₂CH₂NHCOC₆F₅ +·
CH₂CH₂NHCO⁺
m/z 148 (1.0%)
CH₂CH=NH₂⁺
m/z 120 (0.3%)

(cont.)

Fig. 18. Probable mass spectral fragmentation of the PFB derivative of CPPEA.


M⁺·
CH₂CN⁺· OR isomer
m/z 117 (2.0%)
CH₂CH₂⁺ OR CHCH₃
m/z 105 (7.6%)
CH=CH₂⁺·
m/z 104 (20.0%)
CH₂⁺
-CH≡CH
m/z 91 (8.6%)
m/z 65 (1.2%)

8

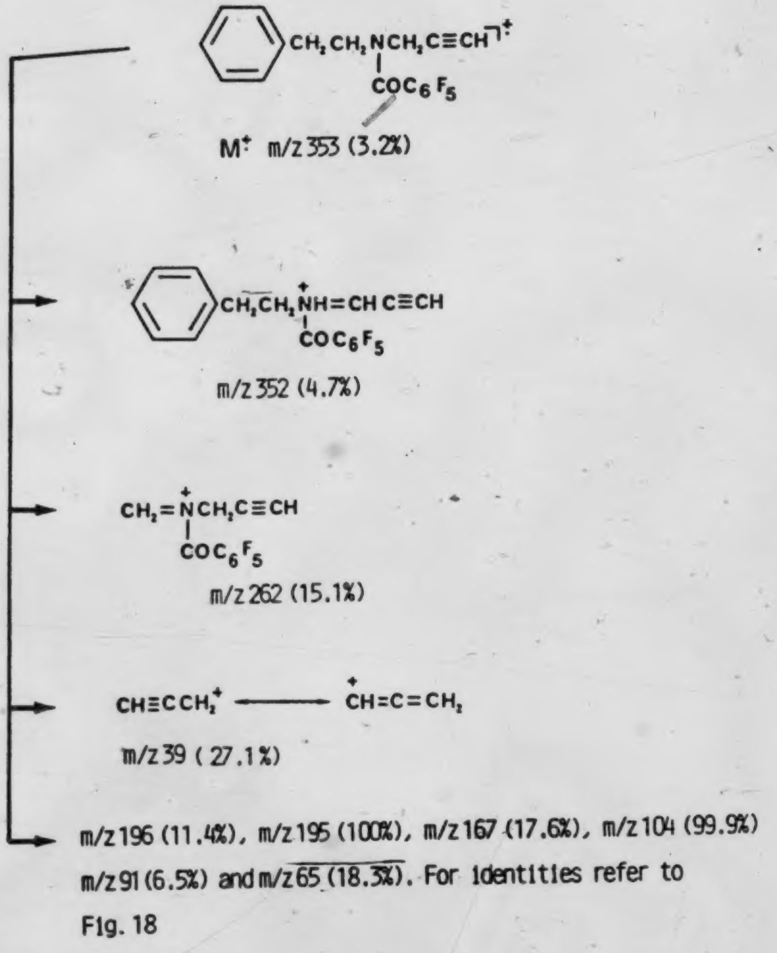

CH₂CH₂NCH₂C≡CH ⁺·
COC₆F₅
M⁺· m/z 353 (3.2%)
CH₂CH₂N⁺H=CH C≡CH
COC₆F₅
m/z 352 (4.7%)
CH₂=N⁺CH₂C≡CH
COC₆F₅
m/z 262 (15.1%)
CH≡CCH₂⁺ ⟷ ⁺CH=C=CH₂
m/z 39 (27.1%)
m/z 196 (11.4%), m/z 195 (100%), m/z 167 (17.6%), m/z 104 (99.9%)
m/z 91 (6.5%) and m/z 65 (18.3%). For identities refer to
Fig. 18

Fig. 19. Probable mass spectral fragmentation of the PFB derivative of PGPEA.

A.3 Analysis of PLZ by aqueous pentafluorobenzoylation

Details of the procedure were given in Section II.D.3. Typical GC traces are presented in Fig. 20. The retention times of mono- and di-derivatives of PLZ and of the derivatized internal standard were 5.2, 6.7 and 5.8 min respectively. The standard curves were linear in the range of 5-1000 ng/3 ml with correlation coefficients r > 0.99. Structures of the final derivatives were confirmed by the GC-MS system described under Section II.A.2.2, and the mass spectral fragmentations are presented in Figs. 21 and 22.

Many alkylhydrazines (or arylalkylhydrazines) during acylation or alkylation generate two derivatives as a result of derivatization at both the nitrogen atoms (Smith, 1983). Phenelzine also forms two derivatives when reacted with PFBC, as evidenced by chromatographic peaks at 5.2 and 6.7 min, under the conditions described in Section II.D.3 (Fig. 20); these peaks correspond to the mono- and diderivative respectively. The two peaks are in the ratio of 5:95 using the conditions described in II.D.3. The diderivative was utilized for analysis and the response (diderivative/internal standard) was found to be linear (correlation coefficient >.99) over the range 5-1000 ng PLZ in 3 ml of sample. There was no interference from PEA, a metabolite of PLZ, since derivatized PEA preceded, and was separable from, derivatized PLZ in the GC assay procedure.

In order to drive the derivatization reaction to completion, different pH conditions and derivatization times (up to 1 hr), various concentrations of PFBC in the toluene/acetonitrile mixture (1-5 μl/ml) and various solvents (ethyl acetate, benzene, toluene, toluene/acetonitrile) were tested with little success. Sequential addition of derivatizing

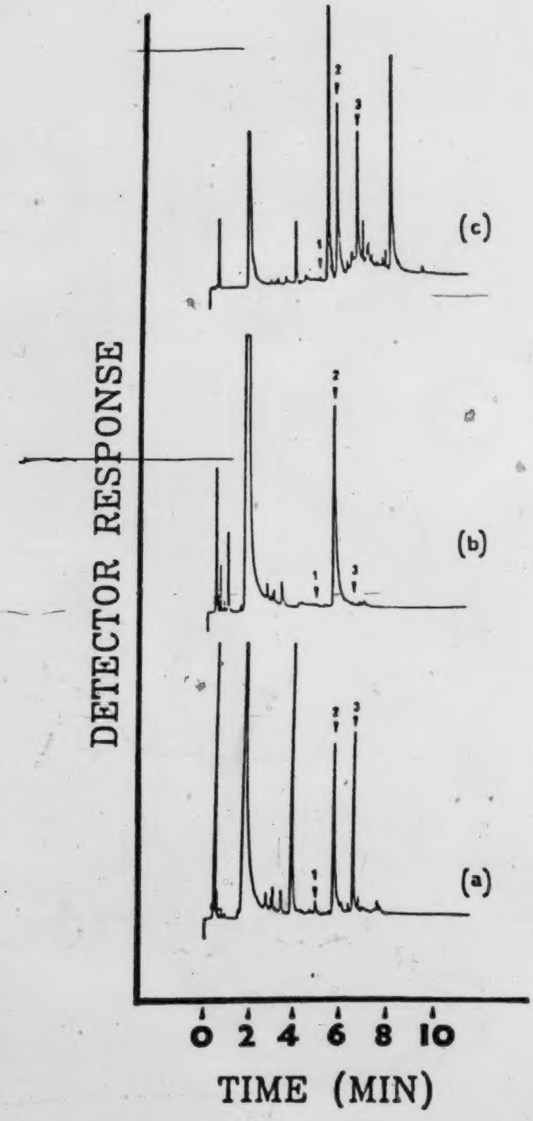


Fig. 20. Typical GC traces from PFBC-treated extracts of: (a) standards of PLZ; (b) 0.4N perchloric acid blank; (c) brain sample from rat treated with PLZ (60 mg/kg, i.p., 4 hr). 1=monoderivative of PLZ, 2=derivatized CPEA (IS), 3=diderivative of PLZ. All the chromatograms were run at an attenuation value of 2♦6.

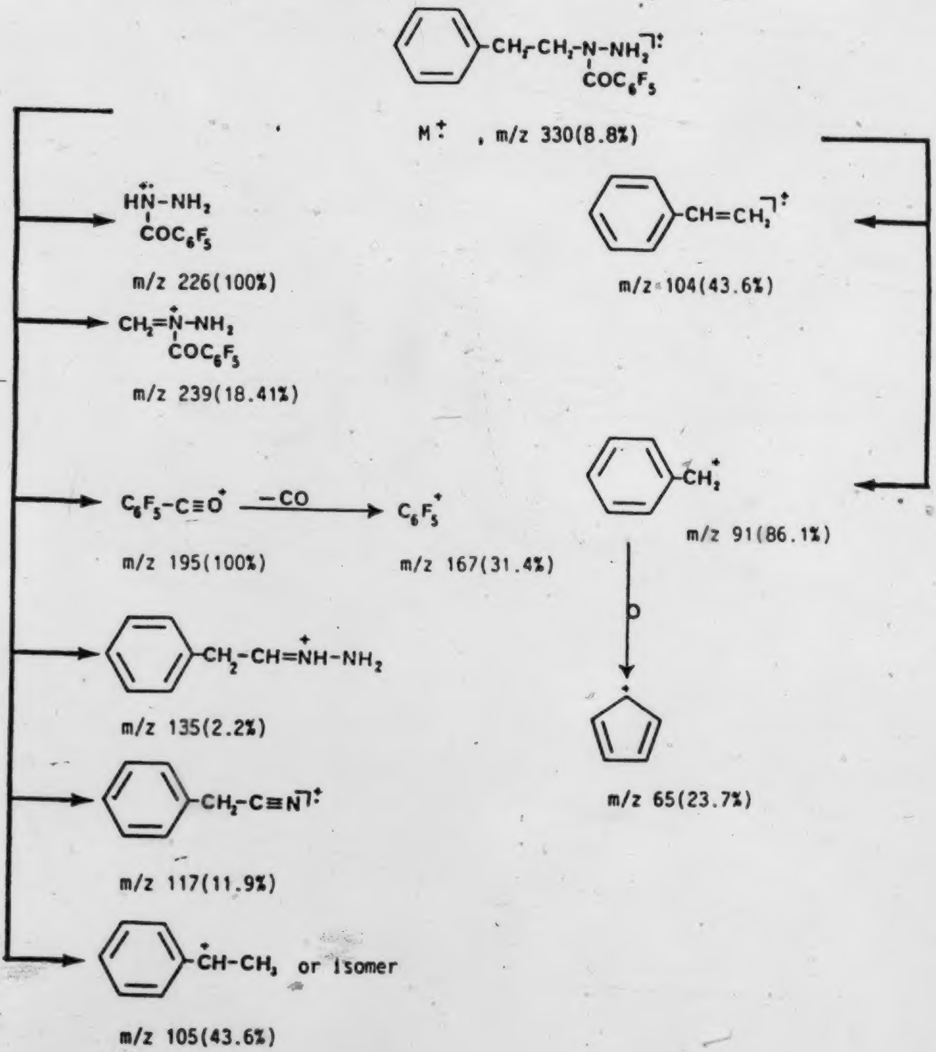

M⁺˙ , m/z 330(8.8%)
m/z 226(100%)
m/z 239(18.41%)
−CO
m/z 195(100%)
m/z 167(31.4%)
m/z 135(2.2%)
m/z 117(11.9%)
or isomer
m/z 105(43.6%)
m/z 104(43.6%)
m/z 91(86.1%)
m/z 65(23.7%)

Fig. 21. Probable mass spectral fragmentation of the mono-PFB derivative of PLZ.

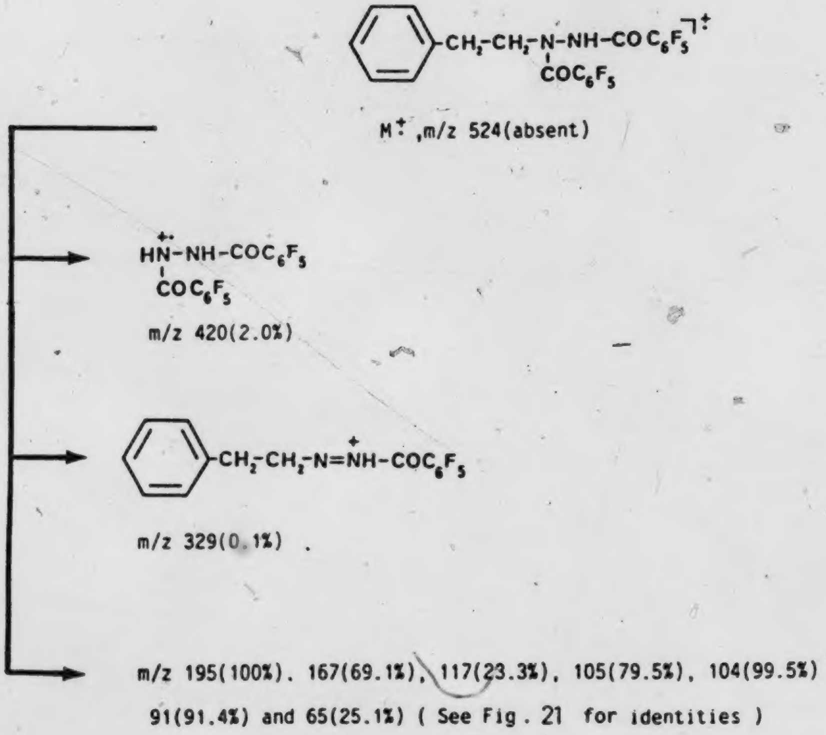

M⁺˙ ,m/z 524(absent)
m/z 420(2.0%)
m/z 329(0.1%)
m/z 195(100%), 167(69.1%), 117(23.3%), 105(79.5%), 104(99.5%)
91(91.4%) and 65(25.1%) (See Fig . 21 for identities)

Fig. 22. Probable mass spectral fragmentation of the di-PFB derivative of PLZ.

agent largely solved the problem. Addition of 2 µl of PFBC (in 20 µl toluene) and shaking for a further 10 min after the initial extractive derivatization improved the peak ratio of mono- to diderivative from 5:95 to 0.7:99.3. (The resultant calibration curves with the diderivative were linear over the concentration range of 5-1000 ng in 3 ml water). The peak ratio of the mono- and diderivatives to each other was relatively unaffected by a wide range of pH values. Samples containing 100 ng of PLZ and 500 ng of internal standard in 3 ml of water were adjusted to pH values of 8.2, 9.0, 10.0, 11.0 or 12.0. The ratio of mono- to diderivative was as follows: 0.7:99.3 at pH 8.2; 1.7:98.3 at pH 9.0; 1.0:99.0 at pH 10.0; 0.8:99.2 at pH 11.0; and 0.8:99.2 at pH 12.0. For the routine derivatization of PLZ in tissue samples and body fluids a pH value of 8.2 was employed. This was attained by adding a small excess of solid $KHCO_3$ to the HCl layer after back-extraction from DEHPA (see Section II.D.3).

The recovery of 100 ng of PLZ standards in 3 ml of sample carried through the entire procedure was 37% (mean of 6 experiments). By eliminating the purification step with the liquid ion-pairing agent DEHPA, the mean extraction efficiency increased to 79.7% (N=6). Although the extraction efficiency through DEHPA and HCl is low, these steps were necessary to clean up urine samples. Even with the relatively low recoveries, recovery is directly proportional to PLZ concentration (Beer's Law is obeyed) and the diderivative provides sensitivity sufficient to analyze low nanogram quantities of PLZ in tissues and biological fluids.

The "on-column sensitivity" of the assay is <10 pg (corrected for recovery), which could be improved 3-4 fold by reducing the volume of toluene in which the derivative was finally dissolved (usually 300 µl) to about 50-100 µl. Intra- and interassay coefficients of variation for 100 ng PLZ standards were determined to be 6.9% (N=22) and 8.7% (N=8) respectively.

A.4 Analysis of PEA using aqueous pentafluorobenzenesulfonylation

The techniques for extraction and analysis were described in Section II.D.4. The retention times of the derivatives of PEA and the internal standard were 5.1 and 5.9 min, respectively, and typical GC traces are presented in Fig. 23. The structure of the derivative was determined by a GC-MS system as described under section II A.2.2; the proposed mass spectral fragmentation is presented in Fig. 24. The standard curves were linear in the concentration range of 1-1000 ng/3 ml.

A.5 Analysis of TCP and FTCP by aqueous pentafluorobenzenesulfonylation

Details of the procedures used for analysis of TCP and its *para*-fluoro analog were presented in Section II.D.5. A typical GC trace is shown in Fig. 25. The standard curves were linear in the range of 2-2000 ng/3 ml. Although GC-ECD was used for routine analysis, the structure of the final derivative was first confirmed by combined GC-MS and the mass spectral fragmentations are presented in Figs. 26 and 27.

A.6 Analysis of T by aqueous pentafluorobenzenesulfonylation

The procedures utilized for extraction and derivatization were given in Section II.D.6. The standard curves were linear in the range of 5-500 ng of T/3 ml sample, with a correlation coefficient of r>0.99.

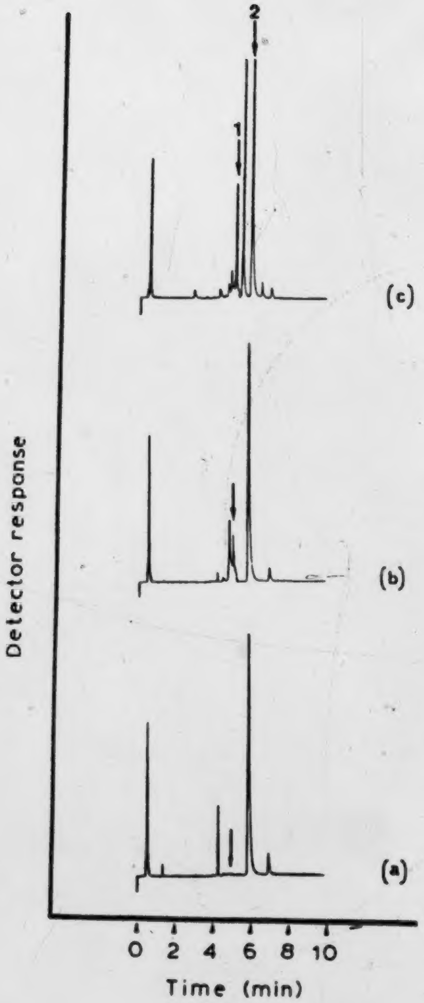


Fig. 23. Typical GC traces from PFBS-treated extracts of (a) 0.1N perchloric acid blank; (b) a control rat brain; and (c) a brain from a rat treated with TCP (0.1 mmol/kg, i.p., 0.5 hr). 1= derivatized PEA, 2=derivatized IS (CPEA). The portion corresponding to peak 1 was run at an attenuation value of 2↓5 in (a) and (b) and 2↓7 in (c).

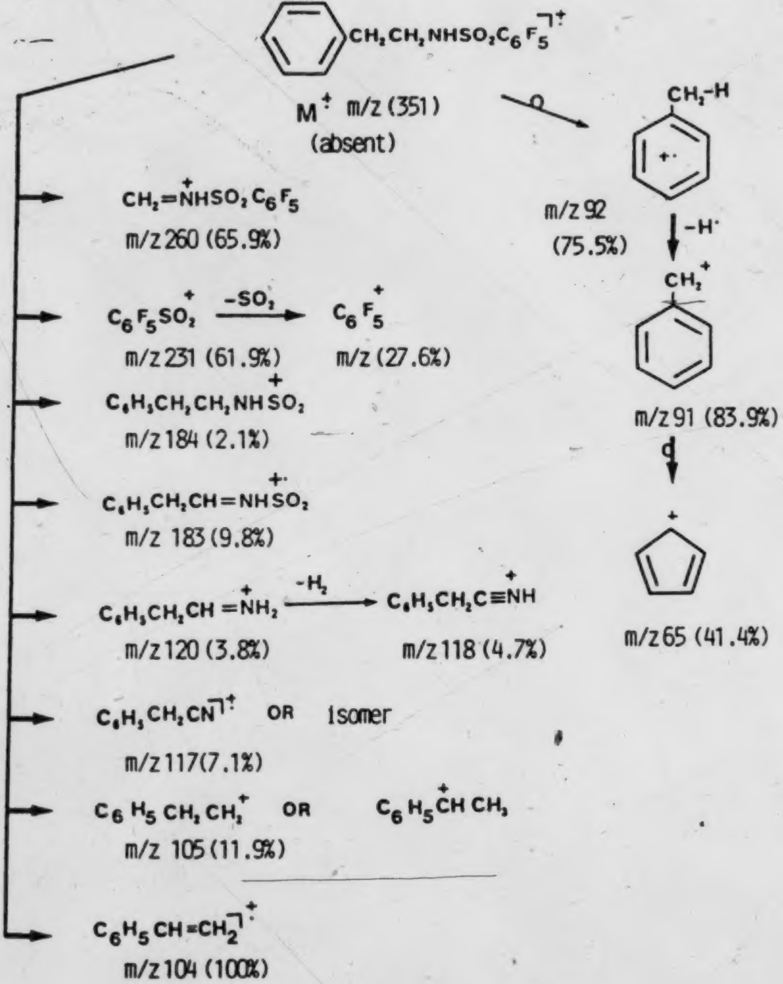

CH₂CH₂NHSO₂C₆F₅ ⁺·
M⁺· m/z (351)
(absent)
CH₂=N⁺HSO₂C₆F₅
m/z 260 (65.9%)
C₆F₅SO₂⁺
-SO₂
C₆F₅⁺
m/z 231 (61.9%)
m/z (27.6%)
C₆H₅CH₂CH₂NHSO₂⁺
m/z 184 (2.1%)
C₆H₅CH₂CH=NHSO₂⁺·
m/z 183 (9.8%)
C₆H₅CH₂CH=N⁺H₂
-H₂
C₆H₅CH₂C≡N⁺H
m/z 120 (3.8%)
m/z 118 (4.7%)
C₆H₅CH₂CN⁺· OR isomer
m/z 117 (7.1%)
C₆H₅CH₂CH₂⁺ OR C₆H₅C⁺HCH₃
m/z 105 (11.9%)
C₆H₅CH=CH₂⁺·
m/z 104 (100%)
CH₂-H
m/z 92
(75.5%)
-H·
CH₂⁺
m/z 91 (83.9%)
m/z 65 (41.4%)

Fig. 24. Probable mass spectral fragmentation of the PFBS derivative of PEA.

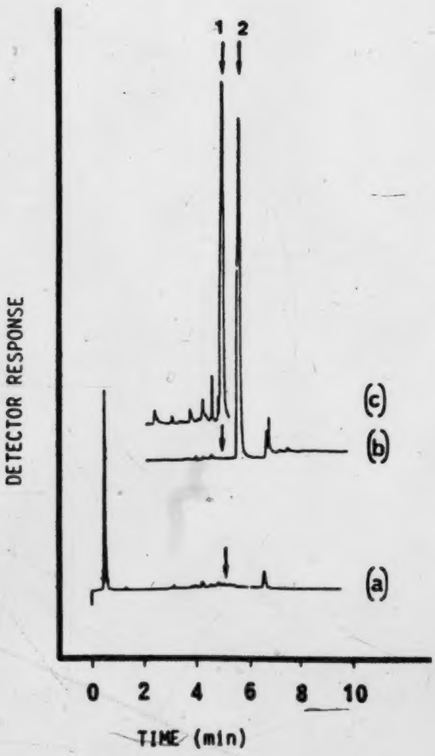


Fig. 25. Typical GC traces of PFBSC-treated extracts of (a) perchloric acid blank; (b) brain from saline-treated rat; (c) brain from rat treated with TCP (0.1 mmol/kg, i.p., 1.5 hr). 1=derivatized TCP, 2=derivatized IS (CPEA). Peak 2 was also present in (c). The attenuation values at the position corresponding to peak 1 are 2↓6 in (a) and (b) and 2↓9 in (c).

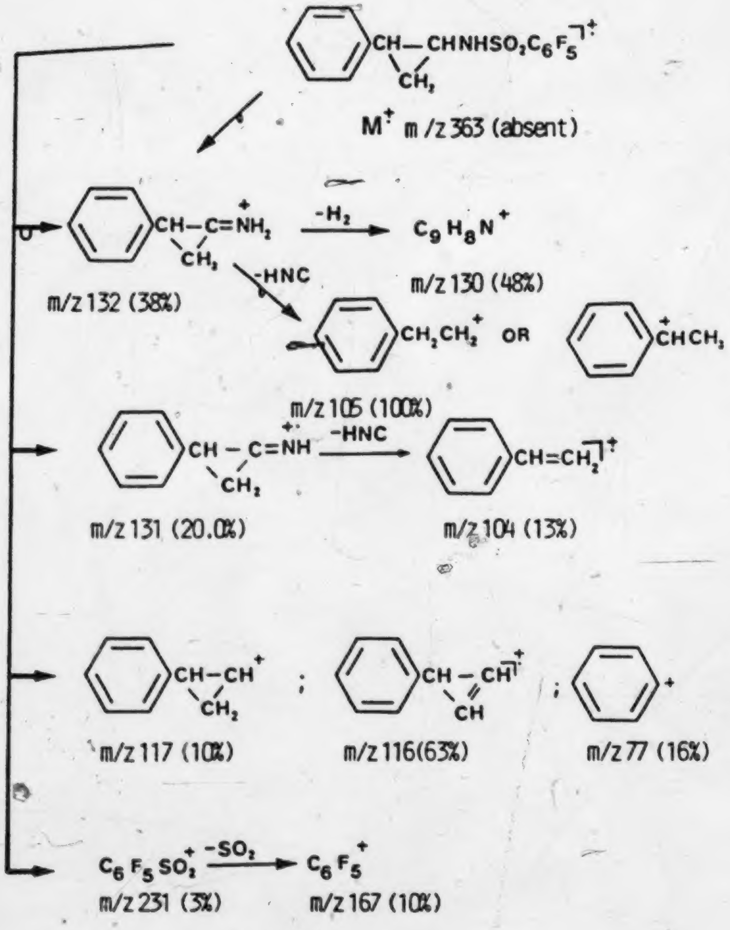
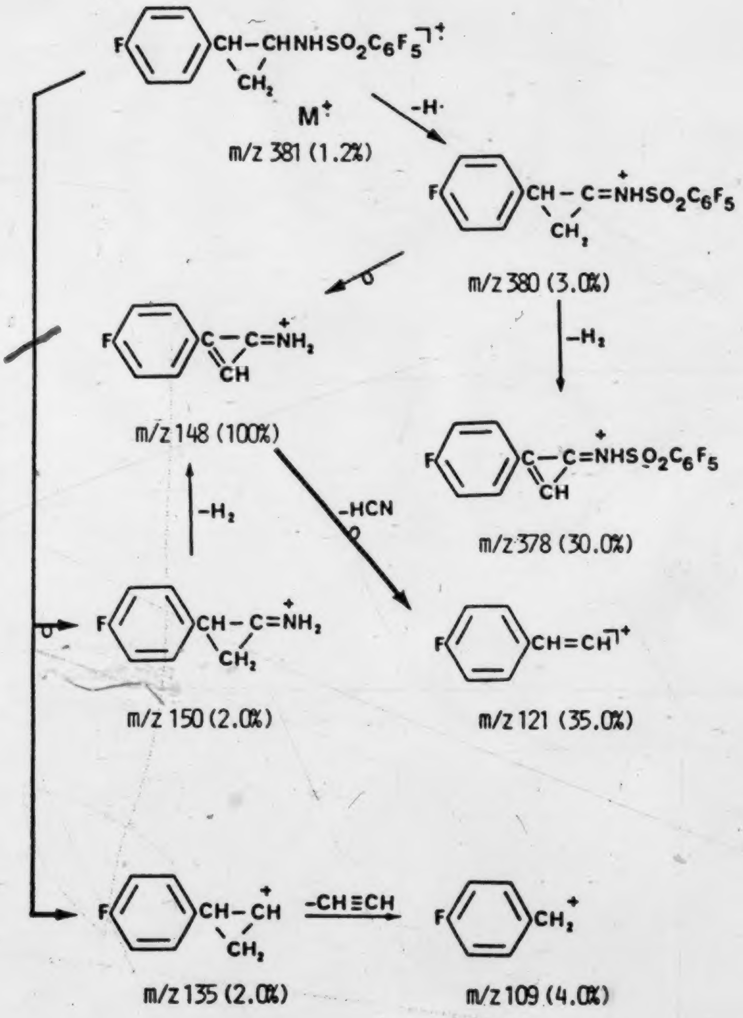
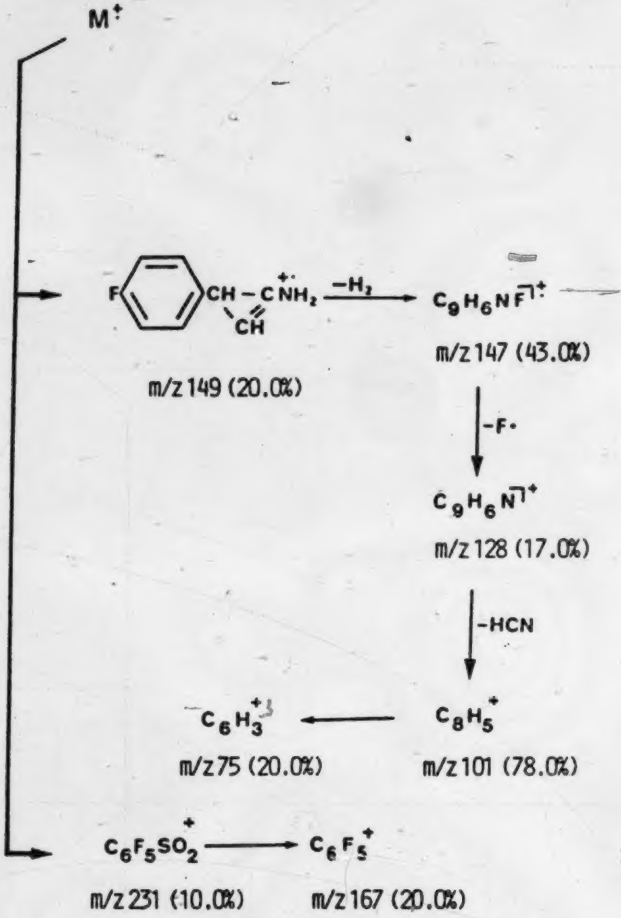

CH—CHNHSO₂C₆F₅
CH₂
M⁺ m/z 363 (absent)
CH—C=NH₂
CH₂
-H₂
C₉H₈N⁺
m/z 132 (38%)
-HNC
m/z 130 (48%)
CH₂CH₂⁺
OR
CHCH₃
m/z 105 (100%)
CH — C=NH
CH₂
-HNC
CH=CH₂
m/z 131 (20.0%)
m/z 104 (13%)
CH—CH
CH₂
CH — CH
CH
m/z 117 (10%)
m/z 116 (63%)
m/z 77 (16%)
C₆F₅SO₂⁺
-SO₂
C₆F₅⁺
m/z 231 (3%)
m/z 167 (10%)

Fig. 26. Probable mass spectral fragmentation of the PFBS derivative of TCP.


M⁺
m/z 381 (1.2%)
-H·
m/z 380 (3.0%)
-H₂
m/z 378 (30.0%)
m/z 148 (100%)
-H₂
-HCN
m/z 150 (2.0%)
m/z 121 (35.0%)
-CH≡CH
m/z 135 (2.0%)
m/z 109 (4.0%)

(cont.)

Fig. 27. Probable mass spectral fragmentation of the PFBS derivative of FTCP.


M+·
CH–CNH2
CH
−H2
C9H6NF]+·
m/z 147 (43.0%)
m/z 149 (20.0%)
−F·
C9H6N]+
m/z 128 (17.0%)
−HCN
C6H3+
C8H5+
m/z 75 (20.0%)
m/z 101 (78.0%)
C6F5SO2+
C6F5+
m/z 231 (10.0%)
m/z 167 (20.0%)

The GC conditions were ~~identical~~ to those mentioned in Sections II.D.4 and II.D.5., and a typical GC trace is illustrated in Fig. 28. The retention times of derivatized T and 5-MT were 7.6 and 7.8 min respectively. Combined GC-MS was used for identification of the structure of the final derivative and the proposed mass spectral fragmentations are presented in Fig. 29.

A.7 Simultaneous analysis of various combinations of trace amines, prodrugs, TCP and TCP analogs

By making slight modifications of the pentafluorobenzenesulfonylation techniques described previously for PEA, T, TCP and FTCP, it was possible to analyze several combinations of the drugs and amines. These modifications were described in Sections II.D.7 to II.D.9.

The utilization of CPEA as internal standard resulted in simultaneous analysis of PEA, TCP (or FTCP) and T. Retention times were: PEA (5.1 min), TCP (5.4 min), FTCP (5.4 min), T (7.6 min) and CPEA (5.9 min). As described in Section II.D.8, alteration in the derivatization time resulted in simultaneous analysis of T and CET (retention times of 7.6 and 9.4 min). In keeping with previous studies, GC-MS analysis was used for confirmation of the structure of derivatized CET; the proposed mass fragmentation pattern is shown in Fig. 30.

By changing the internal standard to BZA and increasing the reaction time (Section II.D.9), the pentafluorobenzenesulfonylation procedure permitted simultaneous analysis of PEA, T, TCP and PGTCP, with retention times of the derivatives being 4.6, 5.0, 5.3, 5.6 and 7.5 min respectively. The proposed mass spectral fragmentations for derivatized PGTCP are illustrated in Fig. 31.

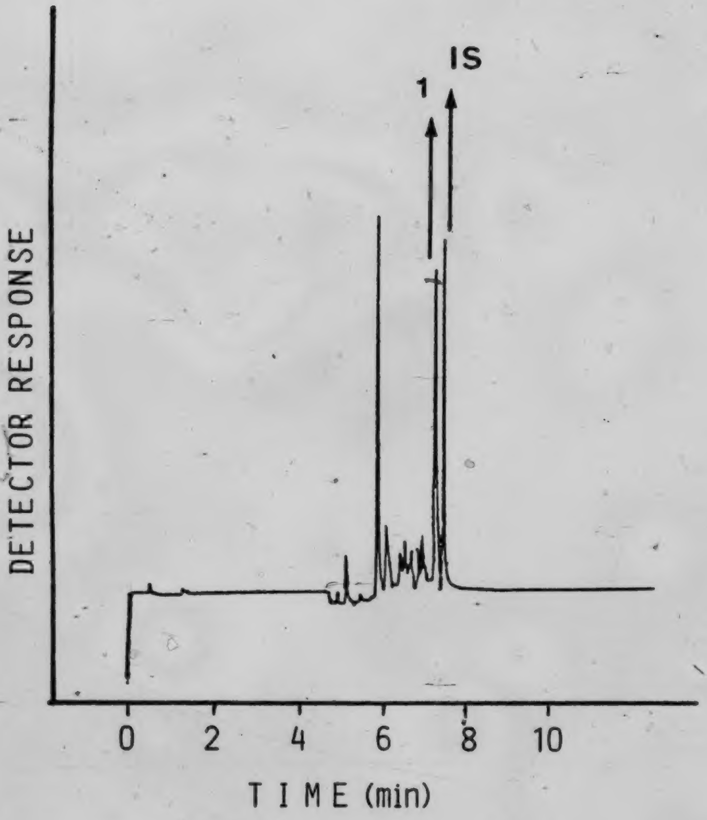
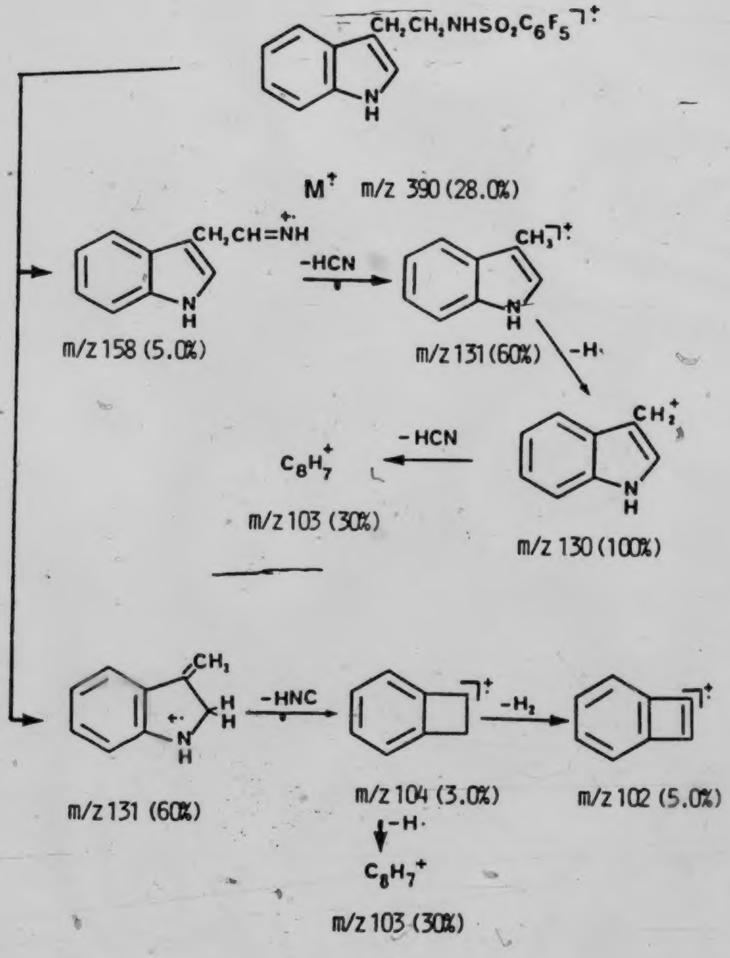
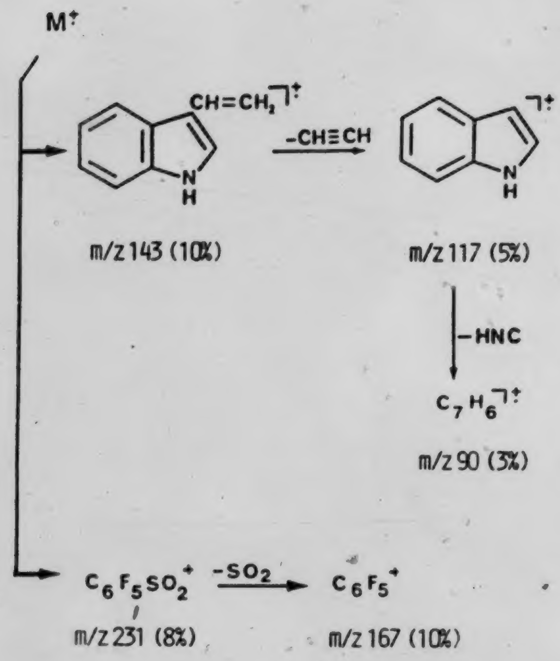


Fig. 28. A GC trace of a PFBSC-treated extract from a rat injected with T (0.1 mmol/kg, i.p., 15 min). The trace was recorded at an attenuation value of 2↑5 for peak 1 (derivatized T) and 2↑7 for IS (derivatized 5-MT).


CH₂CH₂NHSO₂C₆F₅ ⁺·
M⁺· m/z 390 (28.0%)
CH₂CH=NH⁺·
-HCN
CH₃ ⁺·
m/z 158 (5.0%)
m/z 131 (60%)
-H·
CH₂⁺
-HCN
C₈H₇⁺
m/z 103 (30%)
m/z 130 (100%)
CH₃
H
H
-HNC
-H₂
m/z 131 (60%)
m/z 104 (3.0%)
m/z 102 (5.0%)
-H·
C₈H₇⁺
m/z 103 (30%)

(contd.)

Fig. 29. Probable mass spectral fragmentation of the PFBS derivative of T.


M⁺˙
CH=CH₂
-CH≡CH
N H
m/z 143 (10%)
m/z 117 (5%)
-HNC
C₇H₆⁺˙
m/z 90 (3%)
C₆F₅SO₂⁺
-SO₂
C₆F₅⁺
m/z 231 (8%)
m/z 167 (10%)

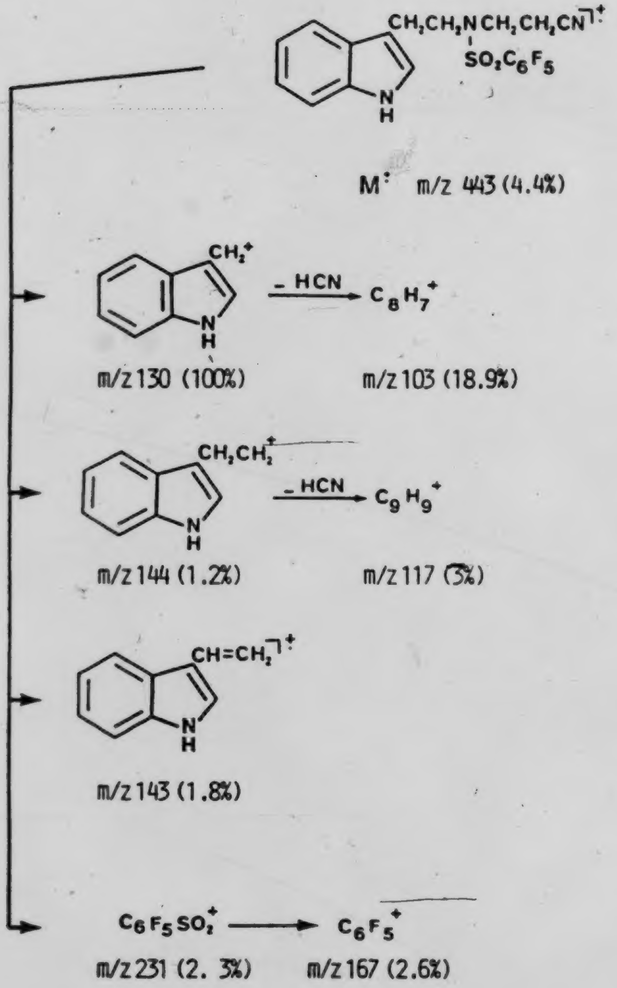

CH2CH2NCH2CH2CN
SO2C6F5
N
H
M+· m/z 443 (4.4%)
CH2+
- HCN
C8H7+
m/z 130 (100%)
m/z 103 (18.9%)
CH2CH2+
- HCN
C9H9+
m/z 144 (1.2%)
m/z 117 (3%)
CH=CH2
m/z 143 (1.8%)
C6F5SO2+
C6F5+
m/z 231 (2.3%)
m/z 167 (2.6%)

Fig. 30. Probable mass spectral fragmentation of the PFBS derivative of CET.

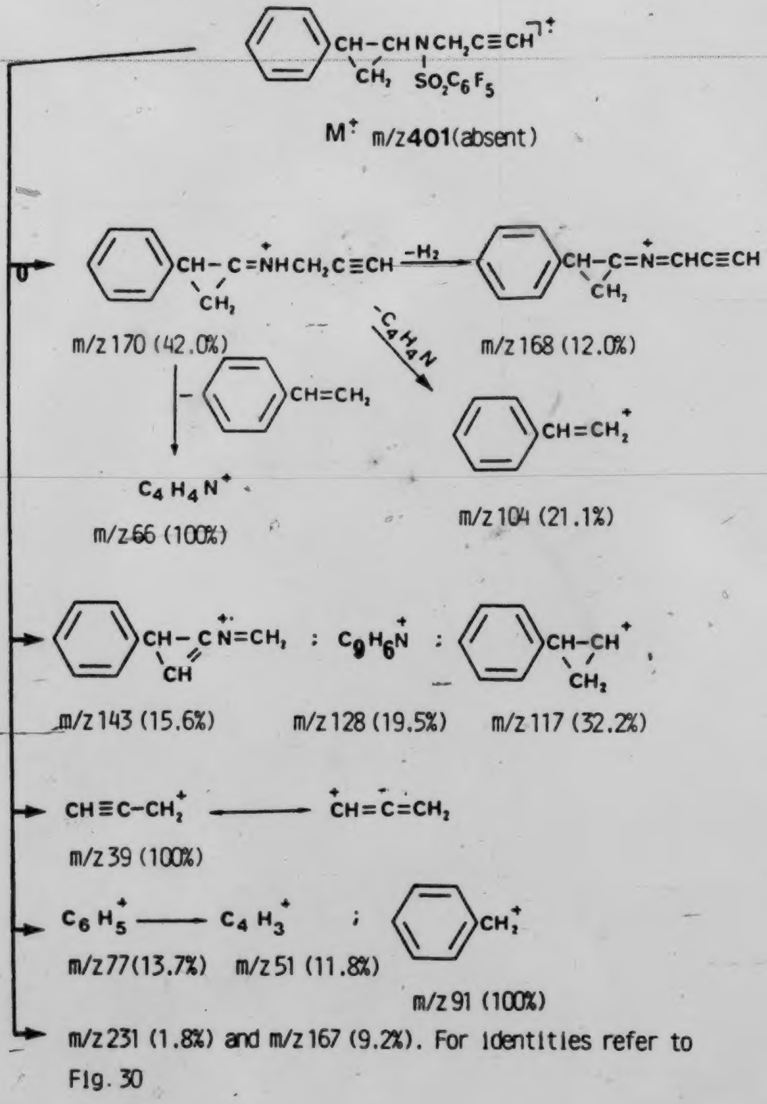

CH-CHNCH2C≡CH
CH2
SO2C6F5
M+ m/z 401 (absent)
CH-C=NHCH2C≡CH
CH2
-H2
CH-C=N=CHC≡CH
CH2
m/z 170 (42.0%)
-C4H4N
m/z 168 (12.0%)
CH=CH2
CH=CH2+
C4H4N+
m/z 104 (21.1%)
m/z 66 (100%)
CH-CN=CH2
CH
C9H6N+
CH-CH+
CH2
m/z 143 (15.6%)
m/z 128 (19.5%)
m/z 117 (32.2%)
CH≡C-CH2+
CH=C=CH2
m/z 39 (100%)
C6H5+
C4H3+
CH2+
m/z 77 (13.7%)
m/z 51 (11.8%)
m/z 91 (100%)
m/z 231 (1.8%) and m/z 167 (9.2%). For identities refer to Fig. 30

Fig. 31. Probable mass spectral fragmentation of the the PFBS derivative of PGTCP.

A.8 Analysis of catecholamines, 5-HT, HVA, DOPAC and 5-HIAA by HPLC-EC

Details of the HPLC apparatus and mobile phases were presented in Section II.A.3.1. A typical HPLC trace obtained from a brain extract is shown in Fig. 32.

B. Rat Brain, Blood and Liver Levels of PEA and its Prodrugs

B. 1 Tissue levels of PEA after administration of PEA

The control levels of PEA (mean ± S.E.M., n=6, ng/g) in untreated rat brain, blood and liver were 2.1 ± 0.28, 2.5 ± 0.23 and 3.5 ± 0.16 respectively. After intraperitoneal administration of PEA (0.1 mmol/kg) tissue levels of PEA had increased dramatically. Peak levels of PEA in all the three tissues were observed at 5 min. In brain, peak levels were nearly 1000 times control levels, and these levels decreased to about 5 times control levels (<10 ng/g) by 15 min and to control levels by 180 min (Fig. 33). The concentrations of PEA decreased in a biexponential manner, with half-life of distribution (α) and half-life of elimination (β) values of 1.1 and 342 min respectively. More than 90% of the available PEA in the brain was eliminated within 15 min. The PEA levels in blood also showed a similar profile (Fig. 34). Peak levels at 5 min were nearly 600 times control values, and these decreased to nearly 20 times control values by 30 min and to control values by 180 min. The half-lives α and β were 3.0 and 69 min respectively. The concentration-time profile in liver was markedly different from those in brain and blood (Fig. 35). Peak levels at 5 min were only 120 times higher than control values and these levels decreased monoexponentially with a half-life of 27 min.

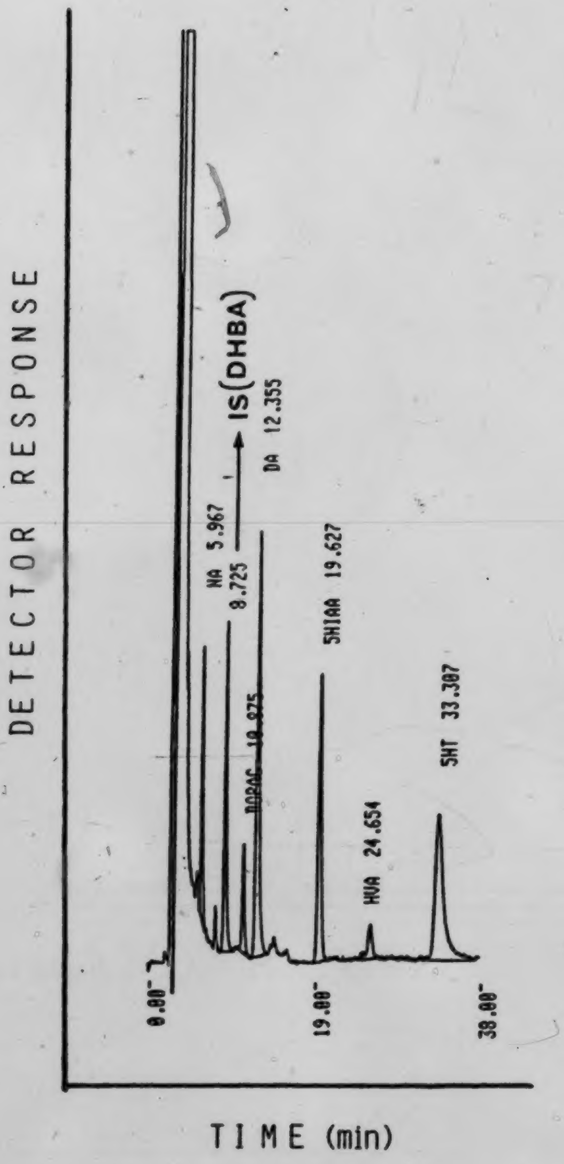


Fig. 32. Typical HPLC trace of an extract of a brain from a saline-treated rat.

Statistical analysis of tissue PEA levels indicated significant between-group differences at different times (i.e., time-dependent changes) in brain [F(7,40)=53.54, P<0.001], blood [F(7,38)=16.95, P<0.001] and liver [F(7,37)=52.39, P<0.001]. Multiple mean comparisons by the Newman-Keuls test indicated that PEA levels in brain and blood at 5 min were significantly different than those at other times. Equivalent tests in liver indicated that PEA levels were increased significantly at 5, 15 and 30 min relative to other times. A comparison of peak levels of PEA in the three tissues indicated no significant difference between brain and blood (P>0.2); there were, however, significant differences between brain and liver (P<0.001) and between blood and liver (P<0.02). There were no differences in the availability of PEA, as measured by the AUC, between brain and liver, brain and blood, and between blood and liver (P>0.1). The pharmacokinetic parameters are summarized in Table 8.

B.2 Tissue levels of PEA and N-(2-cyanoethyl)-2-phenylethylamine (CEPEA) after administration of CEPEA

After administration of CEPEA, tissue levels of PEA increased slowly in contrast to the transient increases seen after PEA injection. In brain, peak levels of nearly 140 times control values were achieved at 15 min and these decreased monoexponentially with a β-value of 22.5 min (Fig. 33). In blood, peak levels (at 15 min) were nearly 130 times control values and these decreased monoexponentially with a β-value of 27 min (Fig. 34). In contrast, peak levels in liver at 15 min were nearly 600 times control values and these decreased monoexponentially with a β-value of 30 min (Fig. 35). Peak levels in liver were significantly higher than those in brain and blood (P<0.01) whereas there was no difference between brain and blood peak levels (P>0.1). There were significant time-dependent changes in brain PEA levels [F(7,40)=34.11, P<0.001] and the multiple mean comparisons revealed that the greatest increases in PEA levels were at 15 min. Levels at 5, 30 and 60 min were also significantly higher than those observed at the other time intervals. Levels of PEA in liver also exhibited time-dependent changes [F(7,38)=53.13, P<0.001]. Levels at 15 min were higher than those at 5 min which in turn were higher than those at 30 min, and levels at these three times were significantly different from those at other times. In blood, significant time-dependent changes in PEA levels were noted [F(7,39)=23.24, P<0.001]. Multiple mean comparisons indicated that levels at 5, 15 and 30 min were significantly different from those at other times. The availability of PEA as measured by AUC was significantly different between liver and brain, liver and blood (P<0.001) but not between brain and blood (P>0.1). In quantitative terms the availability in liver was nearly 6.5 times higher than that in brain and 6.2 times higher than that in blood. The pharmacokinetic parameters of PEA after administration of CEPEA are presented in Table 8.

Comparison of the overall availability of PEA after an equimolar dose of PEA or CEPEA indicated that CEPEA administration caused significantly higher PEA availability (nearly 7 times) in liver (P<0.001) but not in brain or blood (P>0.1). As illustrated in Figs. 33-35, however, the pattern of increase in PEA in both brain and blood was different after CEPEA administration than after PEA administration, with more consistent, relatively sustained elevations being attained.

Levels of CEPEA in brain, blood and liver are presented in Fig. 36. Peak CEPEA levels in blood were attained at 5 min, and these levels

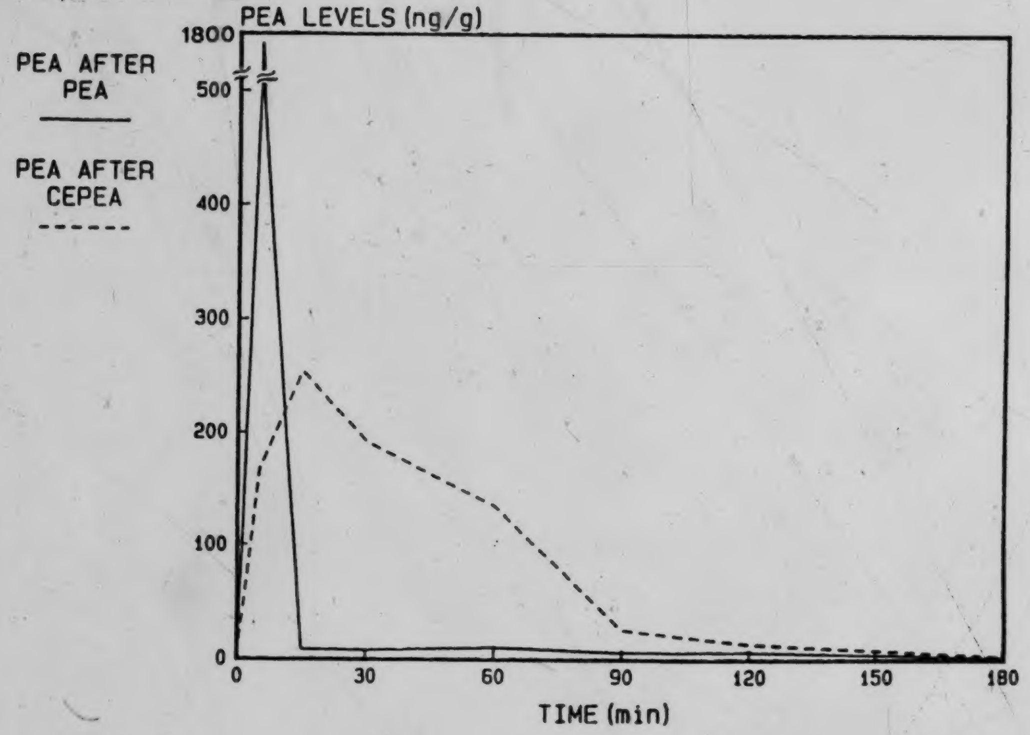


Fig. 33. Rat brain PEA levels (ng/g, n=5-6) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). (S.E.M. values are not represented but they were less than 10% of mean values).

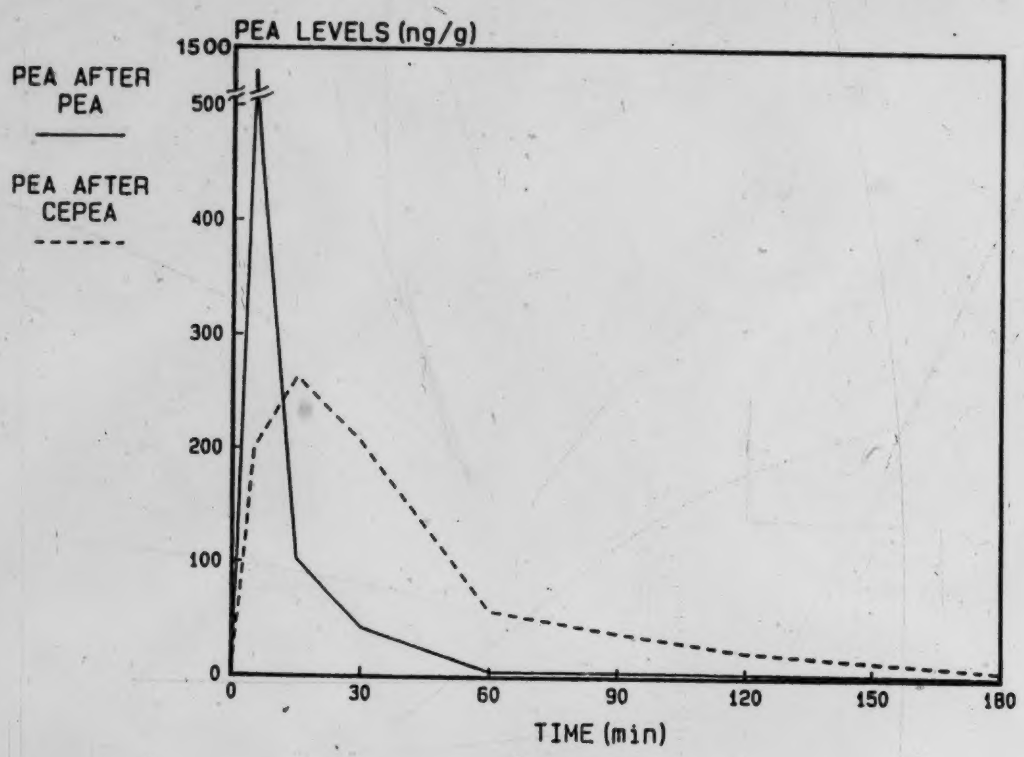


Fig. 34. Rat blood PEA levels (ng/g, n=5-6) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). (S.E.M. values were less than 10% of mean values).

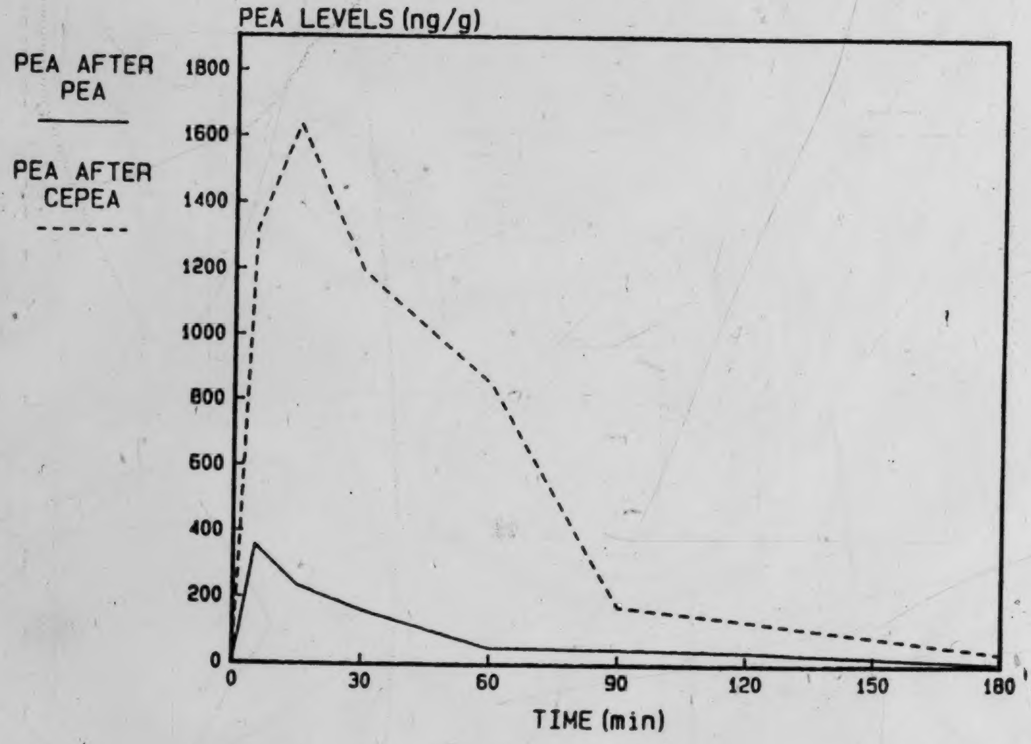


Fig. 35. Rat liver PEA levels (ng/g, n=5-6) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). (S.E.M. values were less than 10% of mean values).

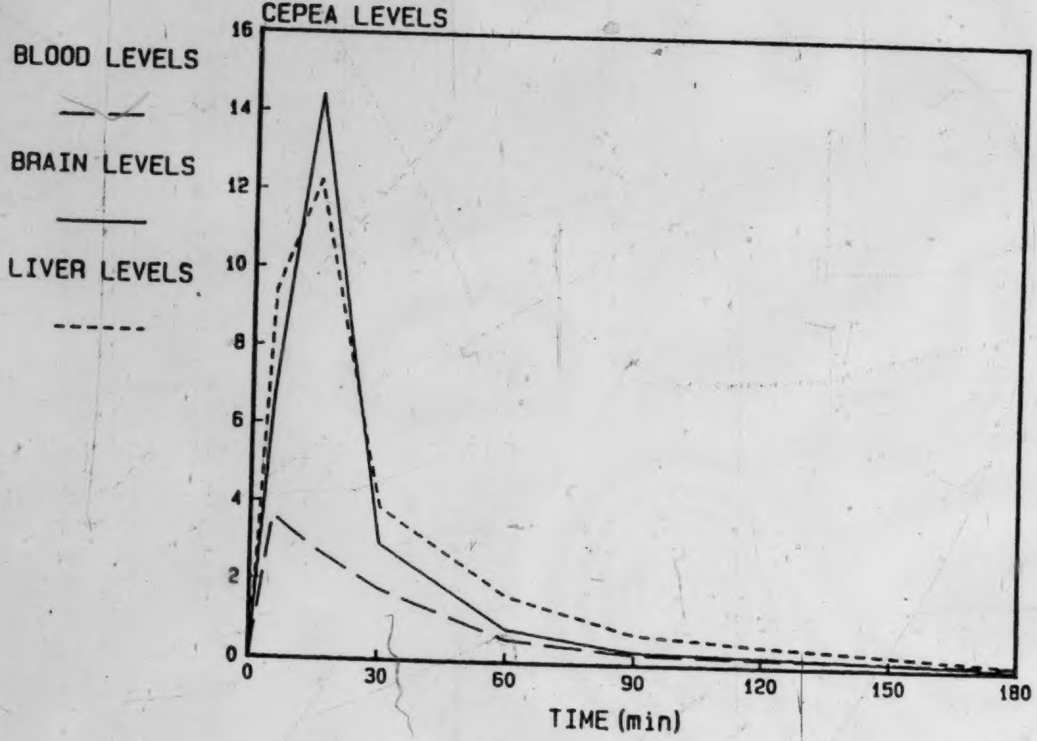


Fig. 36. Rat blood, brain and liver CEPEA levels (μg/g, n=5-6) after administration of CEPEA (0.1 mmol/kg, i.p.). (S.E.M. values were less than 10% of mean values).

decreased biexponentially with half-lives α and β of 8 and 126 min respectively. Peak levels of CEPEA in brain were attained at 15 min and decreased biexponentially with α and β values of 42 and 30 min respectively. The disposition of CEPEA in liver was similar to that in brain, with peak levels attained at 15 min and levels decreasing with α and β values of 4.5 and 35 min respectively. Peak levels of CEPEA in brain and liver were significantly greater than those in blood ($P<0.001$) but there was no difference between brain and liver ($P>0.1$). There were significant time-dependent changes in CEPEA levels in brain [$F(6,35)=50.24$, $P<0.001$], blood [$F(6,35)=41.36$, $P<0.001$] and liver [$F(6,34)=44.98$, $P<0.001$]. The availability of CEPEA as measured by the AUC was significantly different between brain and blood ($P<0.001$), brain and liver ($P<0.005$) and between blood and liver ($P<0.001$). The AUC of CEPEA in liver was 1.2 times that in brain and 3.1 times greater than that in blood. The pharmacokinetic parameters are summarized in Table 8.

A preliminary study was also conducted in which tissue levels of PEA and CEPEA were determined 90 min following 0.1, 0.2 and 0.4 mmol/kg (i.p.) and the results are presented in Table 9. These levels in brain, blood and liver indicated linear increases corresponding to the increases in the doses administered.

B.3 Rat tissue levels of PEA and N-(3-chloropropyl)-2-phenylethylamine (CPPEA) after administration of CPPEA

Semi-logarithmic plots of tissue levels of PEA against time after administration of CPPEA are presented in Fig. 37. Statistical analysis of tissue PEA levels following CPPEA administration indicated significant time-dependent changes in brain [$F(7,40)=41.65$, $P<0.001$), blood [$F(7,41)=35.17$, $P<0.001$] and in liver [$F(7,40)=11.78$, $P<0.001$]; the

Table 8. Pharmacokinetic parameters of PEA and CEPEA in rat tissues following administration of PEA and CEPEA (0.1 mmol/kg, i.p.)

	Tissue	AUC[a] (nmol/g.min)*	Half-life of Distribution (α) (min)	Half-life of Elimination (β) (min)	Cmax[a]
PEA	Blood	102.1 ± 10.2	1.1	342.0	1401 ± 324.8[b]
	Brain	123.0 ± 12.6	3.0	69.0	1775 ± 245.1[b]
	Liver	108.8 ± 16.1	-	27.0	357.1 ± 22.7[b]
PEA after CEPEA	Blood	105.5 ± 8.67	-	27.0	262.5 ± 45.5[b]
	Brain	116.4 ± 7.6	-	22.5	253.6 ± 34.2[b]
	Liver	768.4 ± 48.3	-	30.0	1635 ± 211.8[b]
CEPEA	Blood	741.4 ± 55.8	8.0	126.0	3.6 ± 0.41[c]
	Brain	1954 ± 148.8	4.2	30.0	14.4 ± 1.42[c]
	Liver	2329 + 51.7	4.5	35.0	12.2 ± 1.30[c]

[a]=mean ± S.E.M., n=5-6
[b]=ng/g
[c]=μg/g
*=0-180 min

Table 9. Rat brain, blood and liver PEA and CEPEA levels 90 min after CEPEA administration.

Dose of CEPEA (mmol/kg)	PEA[a] Brain	PEA[a] Blood	PEA[a] Liver	CEPEA[b] Brain	CEPEA[b] Blood	CEPEA[b] Liver
0.1	24.9 ± 4.5	31.3 ± 5.5	171.5 ± 26.4	0.30 ± 0.06	0.15 ± 0.02	0.73 ± 0.09
0.2	61.5 ± 9.7	56.0 ± 6.5	363.8 ± 27.4	0.86 ± 0.06	0.31 ± 0.03	1.4 ± 0.13
0.4	80.8 ± 10.8	150.5 ± 21.9	709.2 ± 99.8	1.6 ± 0.31	0.85 ± 0.11	2.4 ± 0.54

[a]=ng/g, mean ± S.E.M., n=5-6
[b]= μg/g, mean ± S.E.M., n=5-6

results of the multiple mean comparisons are presented in Fig. 37. Peak levels at 15 min in brain, blood and liver were 45, 12 and 40 times the respective control values and these levels decreased biexponentially. The elimination half-life β in liver was nearly 5.1 times and 3.5 times higher than those in blood and brain respectively. Peak levels of PEA in liver were nearly 1.7 and 6 times higher than those in brain ($P<0.02$) and blood ($P<0.001$) respectively. The availability of PEA in liver as measured by the AUC was almost 2.5 and 6 times higher than those in brain ($P<0.001$) and blood ($P<0.001$) respectively. The AUC of PEA in brain was in turn nearly 2.2 times higher than that in blood ($P<0.01$). The pharmacokinetic parameters are summarized in Table 10.

A comparison of the AUC of PEA in brain, blood and liver after administration of an equimolar dose of PEA and CPPEA revealed that the administration of prodrug caused approximately 3 and 6 times lower availability in brain ($P<0.001$) and blood ($P<0.001$) whereas there were no differences in liver after these two treatments ($P>0.1$).

Some interesting differences in the distribution of CPPEA were observed in the three tissues studied (Fig. 38). There were significant time-dependent changes in CPPEA levels in brain [$F(6,35)=22.36$, $P<0.001$], blood [$F(6,35)=8.78$, $P<0.001$] and liver [$F(6,35)=14.68$, $P<0.001$]. Peak levels of CPPEA were observed in liver and blood at 5 min whereas in brain peak levels were observed at 15 min. Peak levels in liver were 2.5 times and 18.5 times higher than those in brain ($P<0.05$) and blood ($P<0.001$) respectively. Tissue CPPEA levels decreased biexponentially in all three tissues and β values in these tissues were similar. Measurements of AUC revealed no significant differences between brain and liver ($P>0.1$), but significant differences

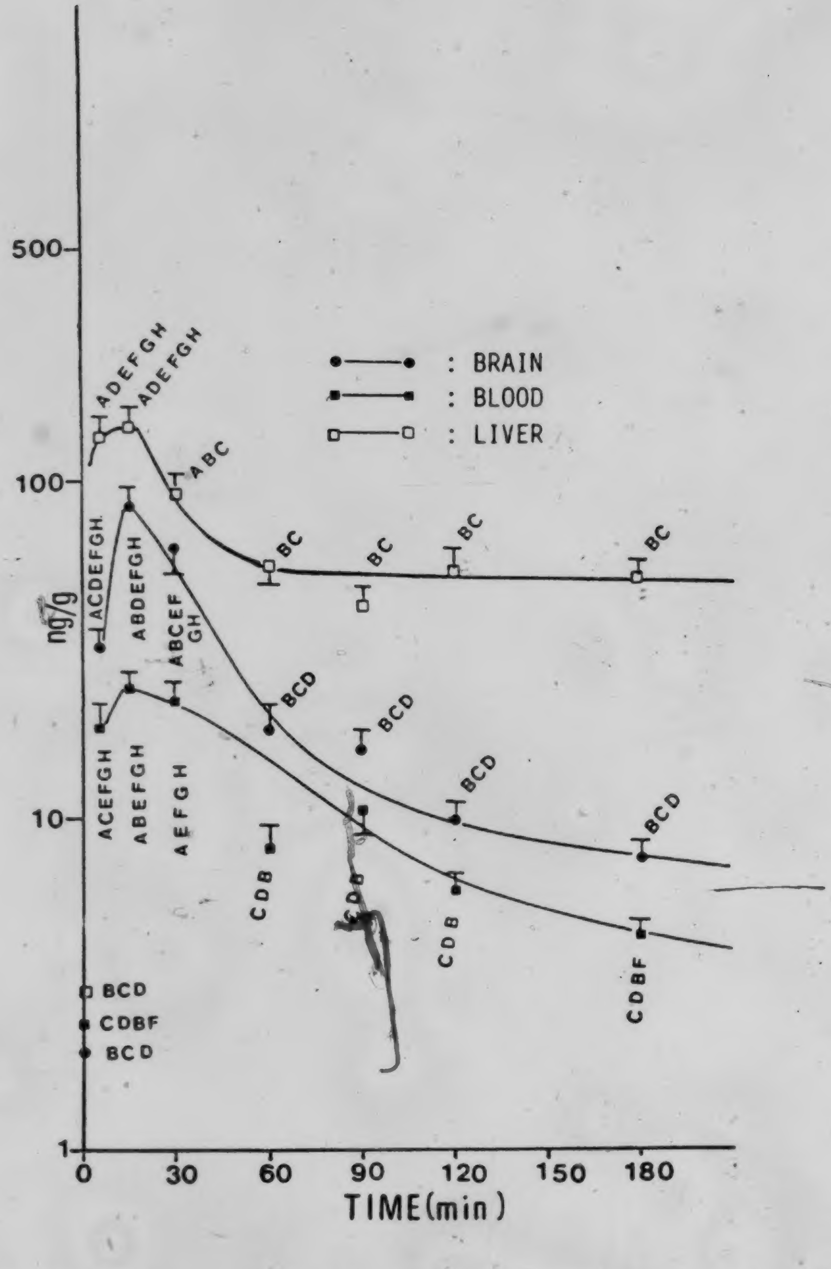


Fig. 37. Rat brain, blood and liver PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of CPPEA (0.1 mmol/kg, i.p.). Superscripts A, B, C, D, E, F, G and H indicate significant differences from levels at 0, 5, 15, 30, 60, 90, 120 and 180 min respectively ($P<0.05$).
]

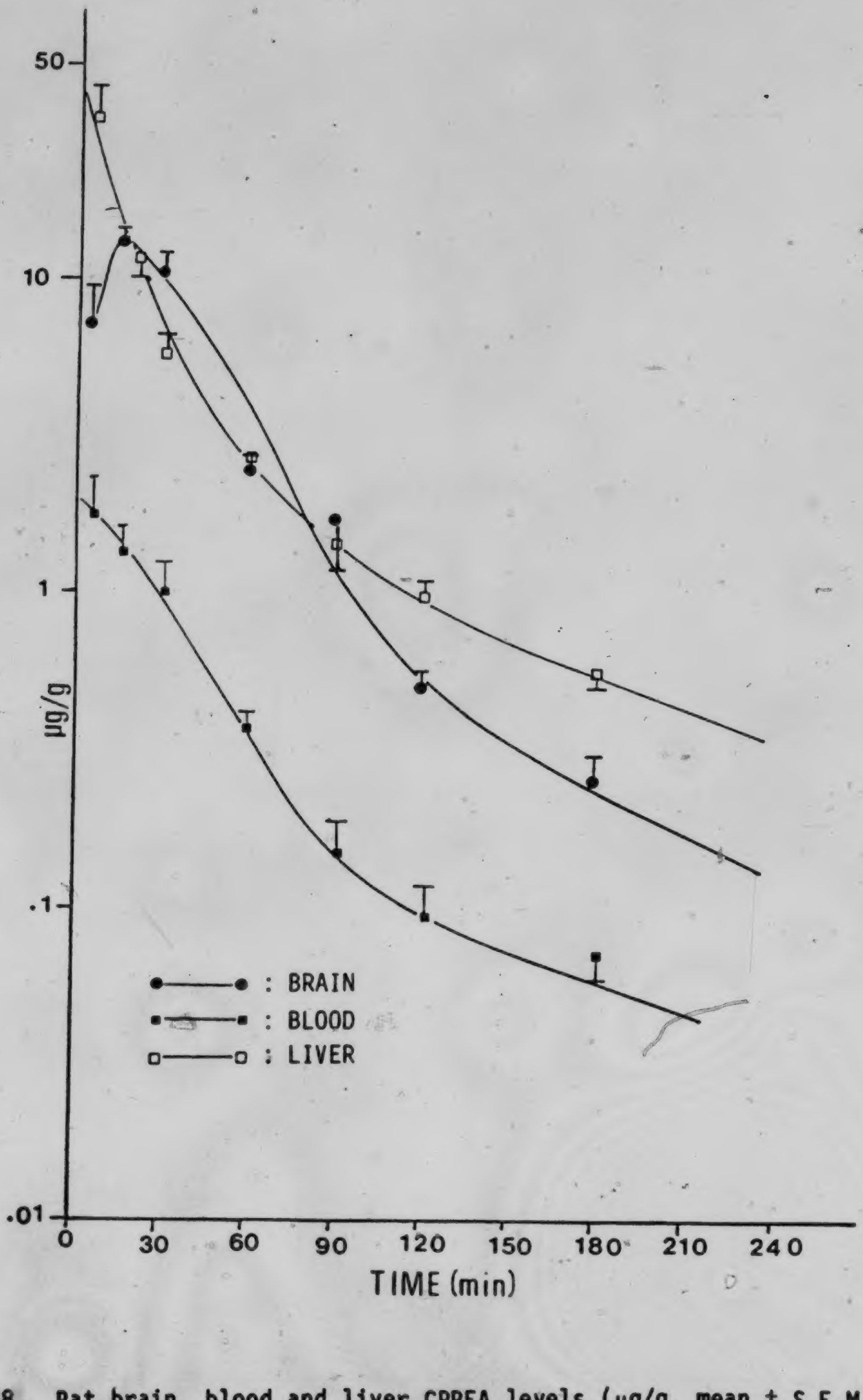


Fig. 38. Rat brain, blood and liver CPPEA levels (μg/g, mean ± S.E.M., n=5-6) after administration of CPPEA (0.1 mmol/kg, i.p.).

Table 10. Pharmacokinetic parameters of PEA and CPPEA in rat tissues following administration of CPPEA (0.1 mmol/kg, i.p.)

	Tissue	AUC[a] (nmol/g.min)*	Half-life of Distribution (α) (min)	Half-life of elimination (β) (min)	C_{max}[a]
PEA after CPPEA	Blood	16.5 ± 0.9	19.5	119.0	25.0 ± 2.5[b]
	Brain	39.7 ± 2.6	7.5	174.0	85.4 ± 12.6[b]
	Liver	99.1 ± 4.3	10.5	607.3	145.0 ± 13.2[b]
CPPEA	Blood	383.0 ± 36.7	13.0	82.0	1.80 ± 0.55[c]
	Brain	3117 ± 253.0	12.5	60.0	13.7 ± 0.70[c]
	Liver	3688 ± 399.7	12.5	87.5	33.2 ± 7.8[c]

[a]=mean ± S.E.M., n=5-6
[b]=ng/g
[c]= μg/g
*=0-180 min

between brain and blood (P<0.001) and between blood and liver (P<0.001) were noted. The pharmacokinetic parameters are summarized in Table 10.

B.4 Rat tissue levels of PEA and N-propargyl-2-phenylethylamine (PGPEA) after administration of PGPEA

Semilogarithmic plots of PEA levels in brain, blood and liver against time are presented in Figs. 39 and 40. Statistical analysis of tissue PEA levels indicated significant time-dependent changes in brain [F(8,42)=14.17, P<0.001), blood [F(8,43)=15.35, P<0.001] and in liver [F(8,43)=14.11, P<0.001]; the results of multiple mean comparisons are presented in Figs. 39 and 40. Administration of PGPEA caused marked increases in tissue PEA levels. In brain, peak levels that were nearly 500 times control values were attained at 30 min and these decreased to nearly 20 times control levels by 240 min. Peak levels of 80 times control levels were attained in blood at 30 min and these levels decreased to nearly 13 times control values by 240 min. In liver, peak levels at 30 min were nearly 1000 times control values and these decreased to approximately 100 times control values by 240 min. Levels in brain, blood and liver were significantly higher than control values even 240 min after administration of PGPEA (P<0.05) and this was in contrast to the rapid decreases in tissue PEA levels observed following an equimolar dose of PEA itself. The levels of PEA after administration of PGPEA decreased biexponentially (Table 11) and the rate of elimination from blood was the slowest among the three tissues studied. There were significant differences in the peak levels of PEA between brain and blood (P<0.01), brain and liver (P<0.01) and between blood and liver (P<0.001). Administration of PGPEA resulted in greater PEA availability in liver than in brain and blood. The AUC of PEA in liver was nearly

2.7 times that in brain ($P<0.001$) which in turn was nearly 4 times the availability in blood ($P<0.001$). A comparison of the availability of PEA (AUC from 0-180 min) following the administration of equimolar doses of PEA and PGPEA showed that PGPEA caused approximately 3.75 times greater PEA availability in brain ($P<0.01$) and nearly 11.5 times greater availability in liver ($P<0.001$) than did PEA itself, while there were no differences in blood ($P>0.05$). The pharmacokinetic parameters are summarized in Table 11.

Levels of PGPEA in brain, blood and liver are presented in Fig. 43. There were significant time-dependent changes in PGPEA levels in brain, blood and liver [$F(7,36)=6.42$, $P<0.001$; $F(7,38)=9.52$, $P<0.001$ and $F(7,39)=14.27$, $P<0.001$ respectively]. Peak levels of PGPEA in liver were nearly 2.5 times higher than those in brain ($P>0.05$) and 7.5 times higher than those in blood ($P<0.01$). Levels of PGPEA in all the three tissues decreased biexponentially and the half-life of elimination in blood was longer than that in brain and liver (a similar trend was seen for its metabolite PEA, see Table 11). The availability of PGPEA in liver was nearly twice the availability in brain ($P<0.001$) and nearly 7 times the availability in blood ($P<0.001$). The pharmacokinetic parameters are summarized in Table 11.

B.5 Rat tissue levels of PEA and PGPEA after administration of N,N-dipropargyl-2-phenylethylamine (DPGPEA)

Semilogarithmic plots of tissue PEA levels against time are presented in Fig. 42. There were significant time-dependent changes in PEA levels in brain [$F(8,41)=14.57$, $P<0.001$], blood [$F(8,40)=3.87$, $P<0.005$] and in liver [$F(8,39)=12.56$, $P<0.001$]; the results of the multiple mean comparisons are presented in Fig. 42. Tissue PEA levels increased

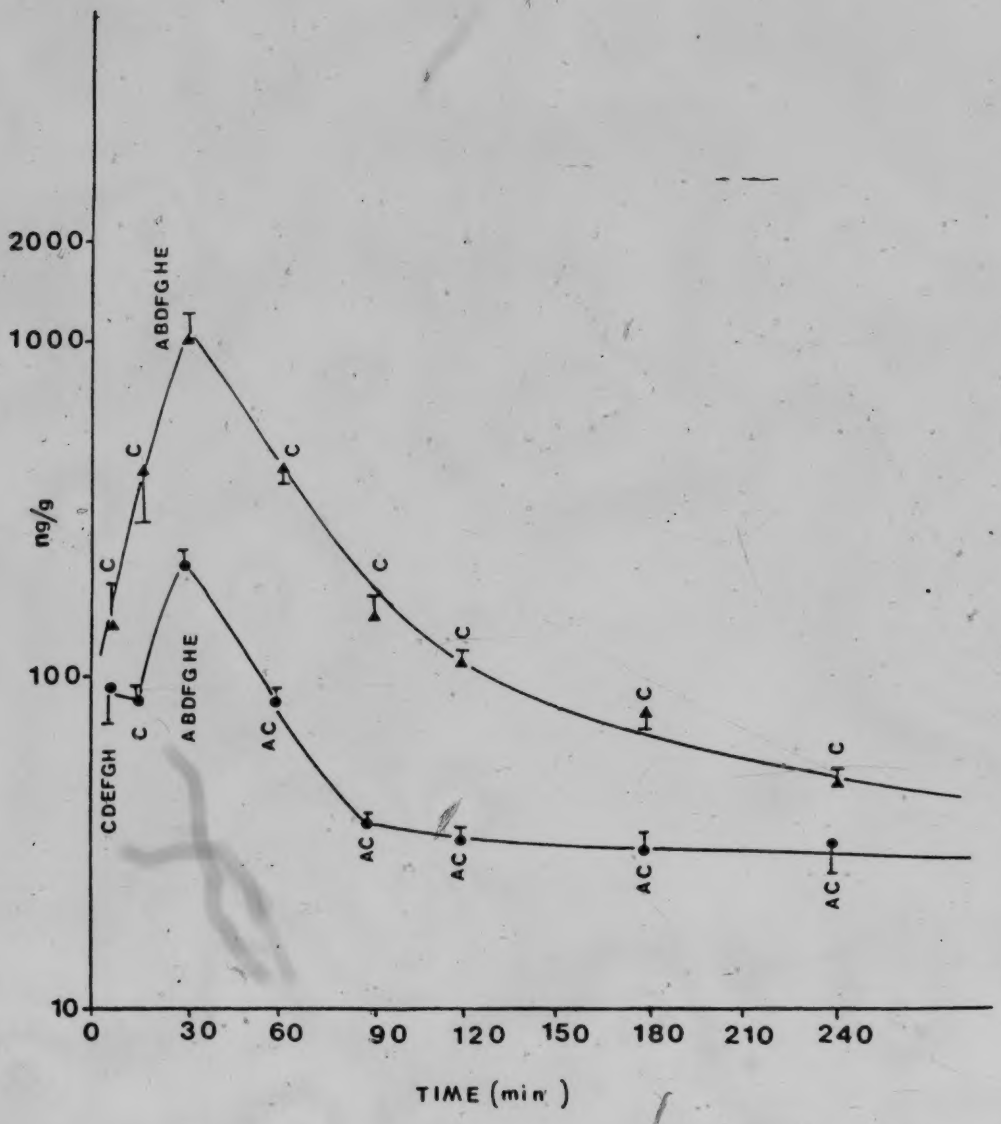


Fig. 39. Rat brain and blood levels of PEA (ng/g, mean ± S.E.M., n=5-6) after administration of PGPEA (0.1 mmol/kg). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 5, 15, 30, 60, 90, 120, 180 and 240 min respectively ($P<0.05$). ▲ =brain, ● =blood.

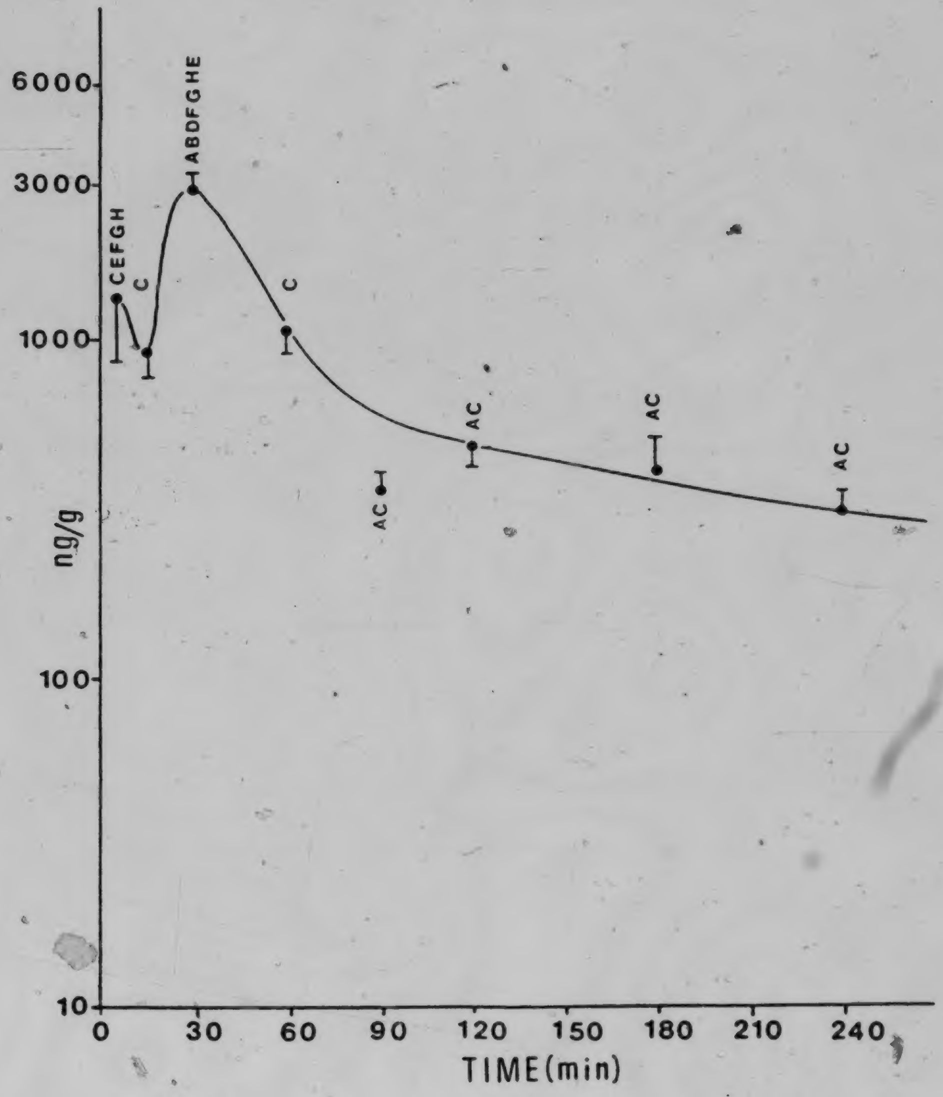


Fig. 40. Liver PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of PGPEA (0.1 mmol/kg, i.p.). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 5, 15, 30, 60, 90, 120, 180 and 240 min respectively ($P<0.05$).

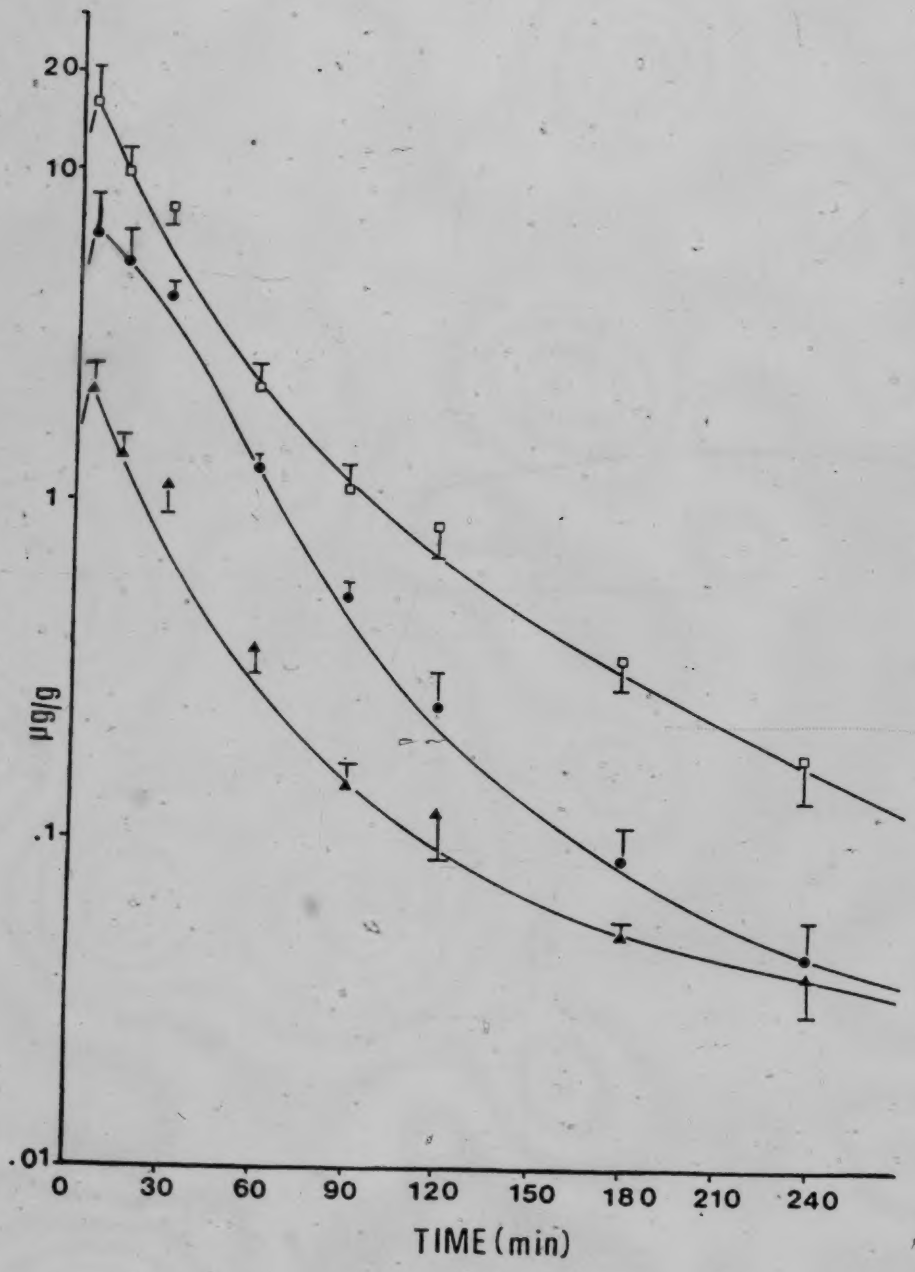


Fig. 41. Rat brain, blood and liver levels of PGPEA (µg/g, mean ± S.E.M., n=5-6) after administration of PGPEA (0.1 mmol/kg, i.p.). ● =brain, ▲ =blood and □ =liver.

Table 11. Pharmacokinetic parameters of PEA and PGPEA in rat tissues after administration of PGPEA (0.1 mmol/kg, i.p.)

	Tissue	AUC[a] (nmol/g.min)*	Half-life of Distribution (α) (min)	Half-life of elimination (β) (min)	C_{max}[a]
PEA after PGPEA	Brain	461.3 ± 49.2	13.5	135.0	1026 ± 201.3[b]
	Blood	109.5 ± 6.2	10.0	467.0	217.2 ± 24.3[b]
	Liver	1245 ± 67.9	7.5	197.5	2937 ± 273.1[b]
PGPEA	Brain	1769 ± 20.6	17.5	75.0	6.4 ± 2.1[c]
	Blood	520.2 ± 67.1	12.5	130.0	2.2 ± 0.61[c]
	Liver	3535 ± 261.9	17.0	62.5	16.1 ± 4.0[c]

[a]=mean ± S.E.M., n=5-6
[b]=ng/g
[c]=μg/g
*=0-180 min

slowly but significantly above the control levels following the administration of DPGPEA. In brain, peak levels of nearly 140 times control values were attained at 60 min and decreased monoexponentially; by 4 hr the levels were approximately 15 times control values (Fig. 42). In blood, peak levels of nearly 35 times control values were attained at 30 min and these decreased monoexponentially to about 6 times control values by 4 hr. The peak PEA levels in liver were nearly 400 times control levels and were reached at 60 min after drug administration, these decreased to nearly 70 times control values by 4 hr. There were significant differences in peak PEA levels between brain and liver ($P<0.001$), brain and blood ($P<0.01$) and between blood and liver ($P<0.001$). The elimination rate of PEA from blood and liver was about half that in brain (Table 12). There were significant differences in the availability of PEA (as measured by AUC) between brain and blood ($P<0.001$), brain and liver ($P<0.001$) and between blood and liver ($P<0.001$). The AUC of PEA in liver was nearly 4 times higher than that in brain and nearly 15 times higher than that in blood. The administration of DPGPEA caused a 2-fold increase in PEA availability to brain ($P<0.001$) and a 9-fold increase in liver ($P<0.001$) relative to the availability estimated after administration of an equimolar dose of PEA itself, whereas in blood the AUC of PEA after PEA administration was significantly higher ($P<0.05$) than that seen after DPGPEA injection.

The levels of a major N-dealkylated metabolite of DPGPEA, namely PGPEA, were also measured in these tissues (Fig. 43). Concentrations of DPGPEA could not be quantitated using the instrumentation available (see Discussion, Section IV.A.5). There were, however, significant time-dependent changes in the levels of PGPEA in brain, blood and liver

[F(7,35)=35.35, P<0.001; F(7,38)=37.54, P<0.001 and F(7,39)=20.75, P<0.001 respectively], and the multiple mean comparisons are presented in Fig. 43. In brain, peak levels of PGPEA were observed at 30 min and these decreased monoexponentially. Peak levels in blood and liver were observed at 5 min and they also decreased monoexponentially (Table 12). The peak PGPEA levels in liver were nearly 5 times higher than those in blood (P<0.001) and nearly 3 times higher than those in brain (P<0.001).

There were significant differences in the availability of PGPEA, as measured by the AUC, between blood and liver (P<0.001), brain and blood (P<0.001) and between brain and liver (P<0.001). The AUC for liver was nearly 8 times higher than that in blood, and nearly twice as high as that in brain. The pharmacokinetic data are summarized in Table 12.

B.6 Comparison of tissue levels of PEA and its prodrugs

Figures 44 and 45 show the comparative availability of PEA and its prodrugs in different tissues after administration of PEA and its prodrugs. Analysis of PEA availability (AUC 0-180 min) after administration of PEA, CEPEA, CPPEA, PGPEA and DPGPEA indicated significant differences between these treatments in brain [F(4,25)=49.60, P<0.001], blood [F(4,25)=28.78, P<0.001] and in liver [F(4,25)=140.21, P<0.001] and the multiple mean comparisons are presented in Fig. 44. Similar analysis of the availability (AUC 0-180 min) of the prodrugs indicated significant differences in brain [F(3,20)=13.62, P<0.01], blood [F(3,20)=10.19, P<0.001] and in liver [F(3,20)=6.07, P<0.005]; the results of the multiple mean comparisons are shown in Fig. 45.

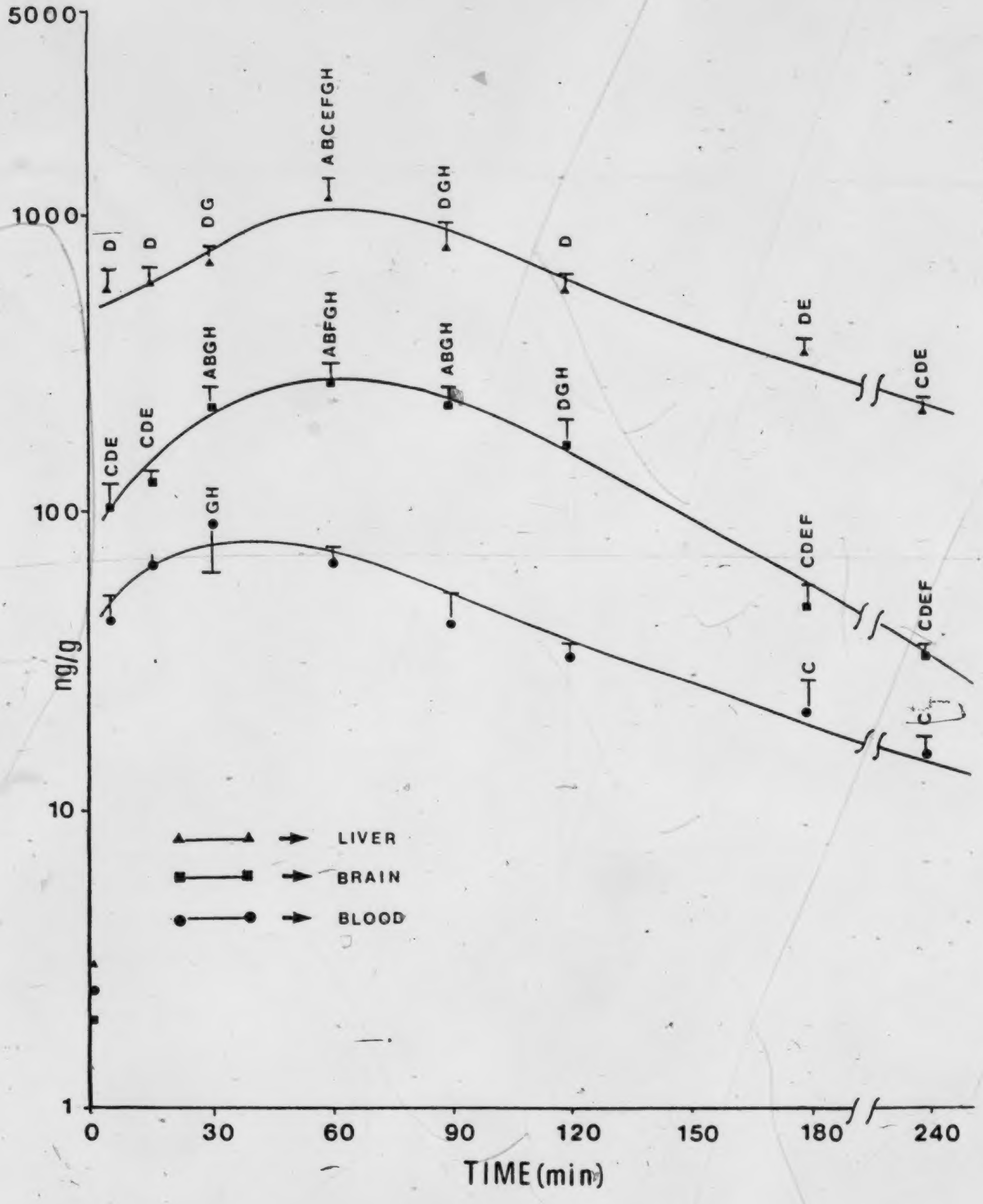


Fig. 42. Rat brain, blood and liver PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of DPGPEA (0.1 mmol/kg, i.p.). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 5, 15, 30, 60, 90, 120, 180 and 240 min respectively (P<0.05).

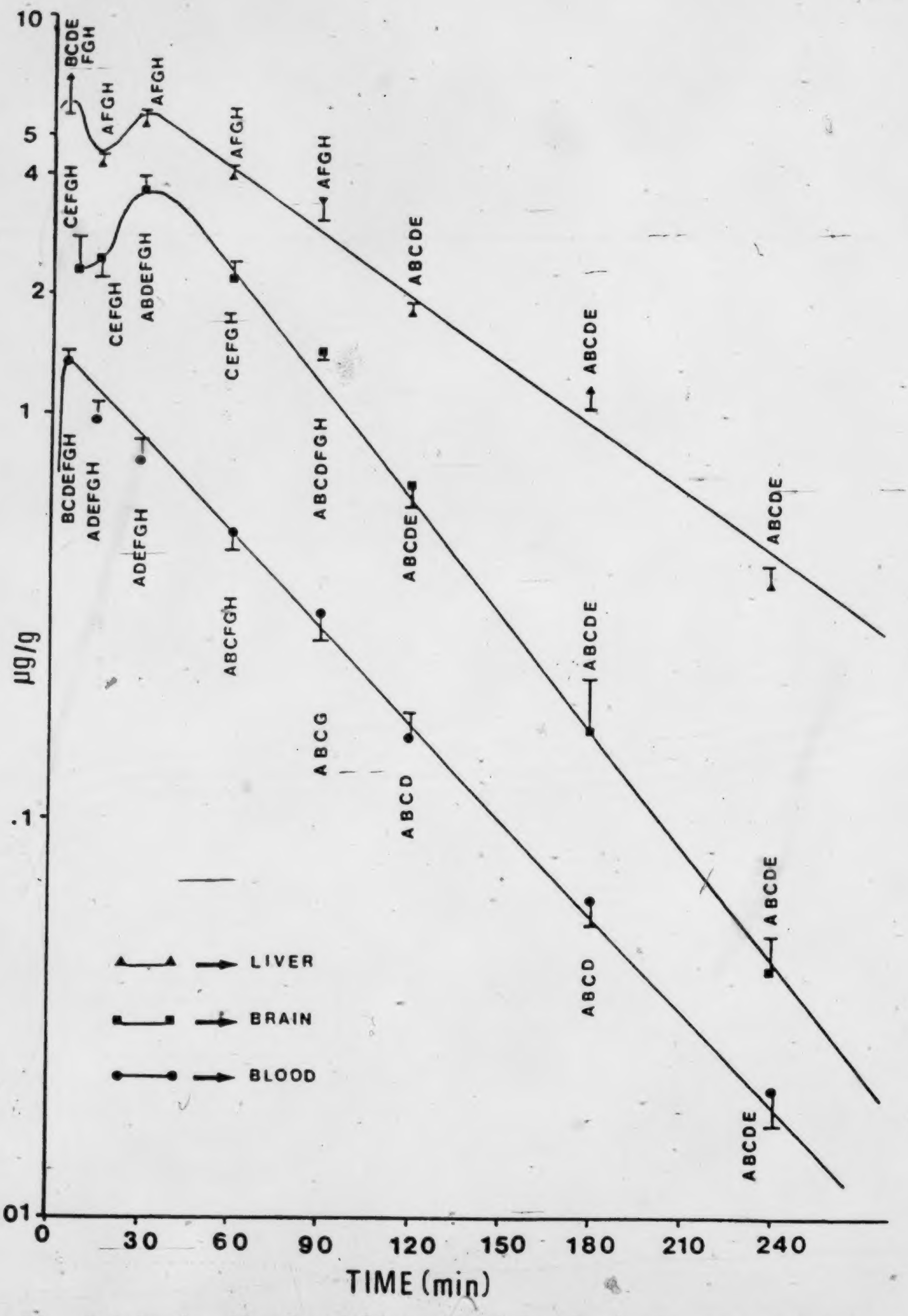


Fig. 43. Rat brain, blood and liver PGPEA levels (µg/g, mean ± S.E.M., n=5-6) after administration of DPGPEA (0.1 mmol/kg, i.p.). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 5, 15, 30, 60, 90, 120, 180 and 240 min respectively ($P<0.05$).

Table 12. Pharmacokinetic parameters of PEA and PGPEA in rat tissues following administration of DPGPEA (0.1 mmol/kg, i.p.)

	Tissue	AUC[a] (nmol/g.min)*	Half-life of elimination (β) (min)	C_{max}[a]
PEA after DPGPEA	Brain	263.2 ± 12.5	48.0	278.1 ± 40.1[b]
	Blood	69.2 ± 6.8	110.0	91.8 ± 30.3[b]
	Liver	1004 ± 49.4	100.0	1177 ± 147.4[b]
PGPEA from DPGPEA	Brain	1684 ± 44.2	32.0	2.4 ± 0.40[c]
	Blood	436 ± 25.4	38.0	1.4 ± 0.11[c]
	Liver	3357 ± 132.1	61.5	6.9 ± 1.03[c]

[a]=mean ± S.E.M., n=5-6
[b]=ng/g
[c]= μg/g
*=0-180 min

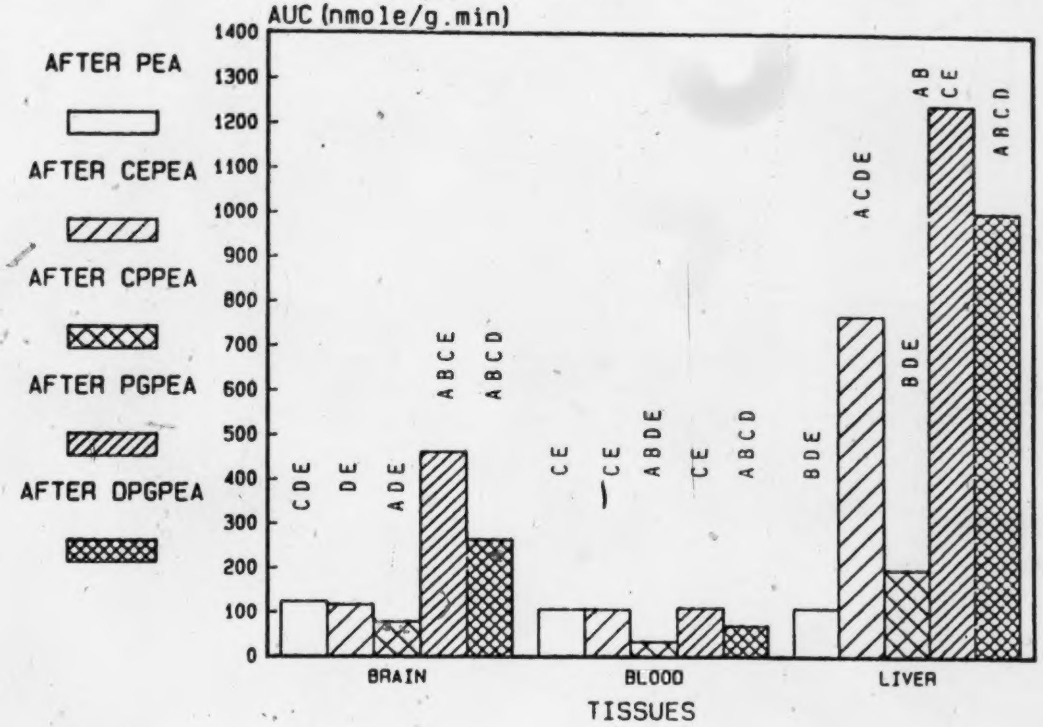


Fig. 44. Comparison of PEA availability (AUC) after administration of PEA, CEPEA, CPPEA, PGPEA and DPGPEA in rat brain, blood and liver. A, B, C, D and E refer to significant differences from AUC values after administration of PEA, CEPEA, CPPEA, PGPEA and DPGPEA respectively.

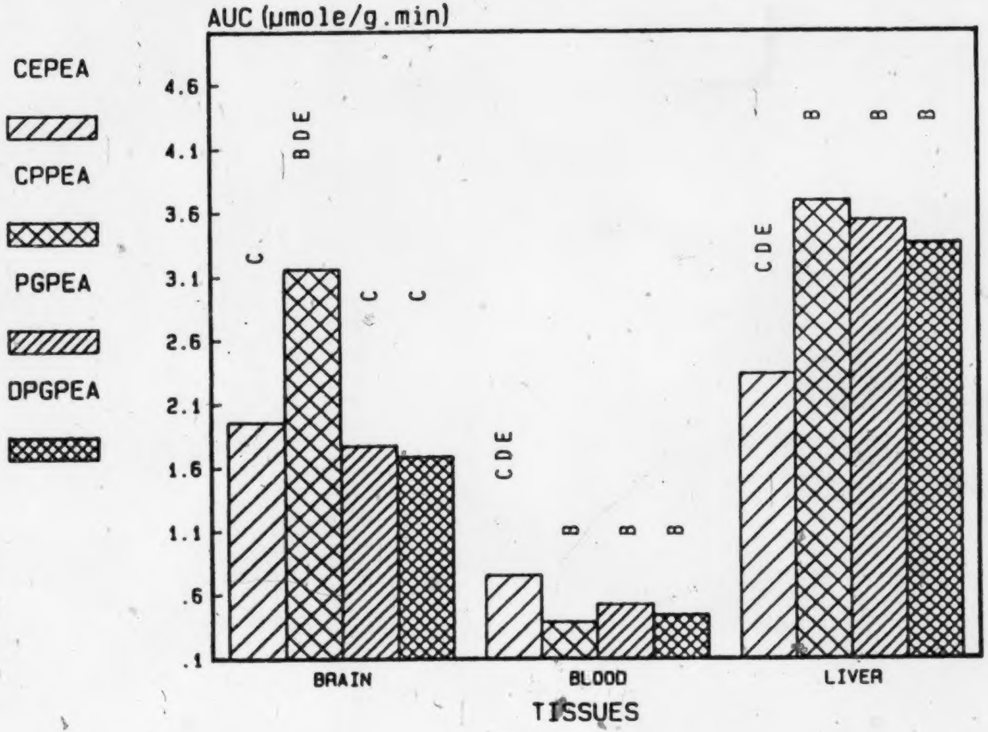


Fig. 45. Comparison of availability (AUC) of prodrugs of PEA in rat brain, blood and liver after administration of 0.1 mmol/kg (i.p.) dose each. For superscripts refer to Fig. 44.

C. Inhibition of MAO Following the Administration of Prodrugs of PEA

C.1 In vivo and in vitro MAO inhibition after administration of CEPEA

In vivo MAO activity was determined in brain at 5, 15, 30, 60 and 120 min following the administration of CEPEA (0.1 mmol/kg, i.p.) and the results are presented in Table 13. From this data it is clear that CEPEA caused weak MAO inhibition; by 120 min the enzyme activity returned to control levels. Enzyme activity at 180 min was not different from control activity. In a preliminary study *in vitro*, the drug (at a concentration of 10 μM) caused 8.4 ± 1.6 and 7.9 ± 1.5% inhibition of MAO-A and -B (mean ± S.E.M., n=4-6) respectively.

C.2 In vivo and in vitro inhibition of MAO by PGPEA and DPGPEA

In vivo inhibition of MAO, using MAO-A- and MAO-B-specific substrates, in brain and liver was determined at 5, 15, 30, 60, 90, 120, 180 and 240 min following the administration of PGPEA and DPGPEA, and the results are summarized in Tables 14 and 15. For comparative purposes brain and liver MAO activities were also determined 60 min following the administration of an equimolar (0.1 mmol/kg, i.p.) dose of TCP, PAR and N-methyl,N-propargyl-2-phenylethylamine (MPGPEA) to other groups of rats, and these data have also been included in Table 14. The inhibition of brain MAO *in vitro* by PGPEA and DPGPEA is shown in Table 16. The *in vitro* data indicated that PGPEA was nearly 3 times more potent at inhibiting MAO-B than MAO-A while DPGPEA was nearly 5 times more potent towards MAO-B than towards MAO-A; PGPEA was approximately 4.7 and 30 times more potent than DPGPEA at inhibiting MAO-A and MAO-B activities respectively. The *in vivo* data indicated that PGPEA administration caused relatively more MAO-B inhibition than MAO-A in brain, while in liver the selectivity was much more pronounced. The MAO inhibitory profile of DPGPEA *in vivo* was similar to that of PGPEA.

D Brain and Liver T Levels Following the Administration of PGPEA

The levels of the trace amine T were determined in brain and liver following the administration of PGPEA, and the levels are presented in Fig. 46. With the present assay method (aqueous pentafluorobenzenesulfonylation) the control levels could not be accurately quantitated, but they were less than 1 ng/g. Using a high resolution mass spectrometric method, Philips *et al.* (1974) reported levels of 0.5 and 0.7 ng/g in brain and liver respectively, and these were used as reference control values in the present work. Although T levels were well above control levels, there were no significant time-dependent changes in T levels in brain and liver [$F(7,29)=0.54$, $P>0.1$ and $F(7,34)=1.16$, $P>0.1$ respectively].

E. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA

E.1 Levels of NA, DA, DOPAC, HVA, 5-HT, 5-HIAA following administration of PEA

In order to investigate the effects of the prodrugs and the altered levels of PEA on neurotransmitter amines, brain concentrations of NA, DA, 5-HT and the metabolites DOPAC, HVA and 5-HIAA were measured in the brains of the drug-treated rats. The results are shown in Figs. 47- 52 where the values of each neurotransmitter or metabolite is expressed as a percentage of values obtained from brains of rats that

Table 13. Inhibition of rat brain MAO after administration of CEPEA (0.1 mmol/g, i.p.) (%inhibition, mean ± S.E.M., n=5-6).

Time (min)	% Inhibition MAO-A	% Inhibition MAO-B
5	9.9 ± 4.8	8.9 ± 1.7
15	5.3 ± 4.5	22.0 ± 2.2
30	11.5 ± 6.6	19.7 ± 4.7
60	0.50 ± 0.30	7.7 ± 2.6
120	3.8 ± 3.7	1.1 ± 0.46

Table 14. *In vivo* inhibition of rat brain and liver MAO following administration of PGPEA, PAR, TCP and MPGPEA (0.1 mmol of each/kg, i.p.)

		% Inhibition[a]			
Drug	Time (min)	Brain		Liver	
		MAO-A	MAO-B	MAO-A	MAO-B
PGPEA	5	33.3 ± 3.0	74.7 ± 1.7	1.4 ± 0.8	72.1 ± 3.0
	15	38.2 ± 2.9	76.9 ± 0.8	7.2 ± 2.2	76.3 ± 2.5
	30	28.7 ± 2.6	74.1 ± 1.1	1.7 ± 0.84	76.8 ± 1.5
	60	23.7 ± 3.1	73.3 ± 1.0	0.1 ± 0.1	71.1 ± 1.3
	90	40.0 ± 1.7	66.5 ± 1.8	4.8 ± 2.1	77.3 ± 0.9
	120	35.5 ± 6.8	58.3 ± 3.5	1.2 ± 1.2	71.9 ± 3.1
	180	38.5 ± 1.9	65.9 ± 1.9	1.7 ± 0.9	79.4 ± 2.9
	240	36.6 ± 2.8	67.2 ± 1.3	6.1 ± 2.1	75.5 ± 1.2
MPGPEA	60	44.3 ± 1.7	79.7 ± 0.7	50.1 ± 2.4	91.5 ± 0.5
PAR	60	93.1 ± 1.1	95.6 ± 1.0	90.9 ± 1.4	94.2 ± 0.9
TCP	60	94.0 ± 1.7	98.1 ± 0.1	96.1 ± 1.1	97.7 ± 0.2

[a]=mean ± S.E.M., n=5-6.

Table 15. Inhibition of rat brain and liver MAO *in vivo* following administration of DPGPEA (0.1 mmol/kg, i.p.).

Time (min)	% Inhibition[a] Brain MAO-A	Brain MAO-B	Liver MAO-A	Liver MAO-B
5	20.5 ± 2.9	60.3 ± 3.7	0.1 ± 0.1	68.2 ± 1.2
15	24.5 ± 3.9	68.7 ± 2.4	0.9 ± 0.8	72.8 + 0.8
30	34.8 ± 4.9	70.4 ± 2.6	0.1 ± 0.1	78.1 ± 1.0
60	27.8 ± 3.3	67.9 ± 2.8	1.7 ± 0.9	78.3 ± 0.7
90	23.8 ± 4.9	68.3 ± 2.2	5.4 ± 2.2	77.3 ± 2.1
120	34.9 ± 4.1	70.1 ± 1.4	5.5 ± 1.5	74.4 ± 1.6
180	28.9 ± 7.9	64.5 ± 2.8	3.2 ± 1.4	71.2 ± 1.4
240	21.4 ± 4.8	64.6 ± 2.0	0.6 ± 0.3	71.6 ± 0.8

[a]-mean ± S.E.M., n=5-6.

Table 16. Inhibition of rat brain MAO *in vitro* by PGPEA and DPGPEA.

	IC_{50} values (M)	
	MAO-A	MAO-B
PGPEA	8.9×10^{-5}	2.6×10^{-6}
DPGPEA	4.2×10^{-4}	7.8×10^{-5}

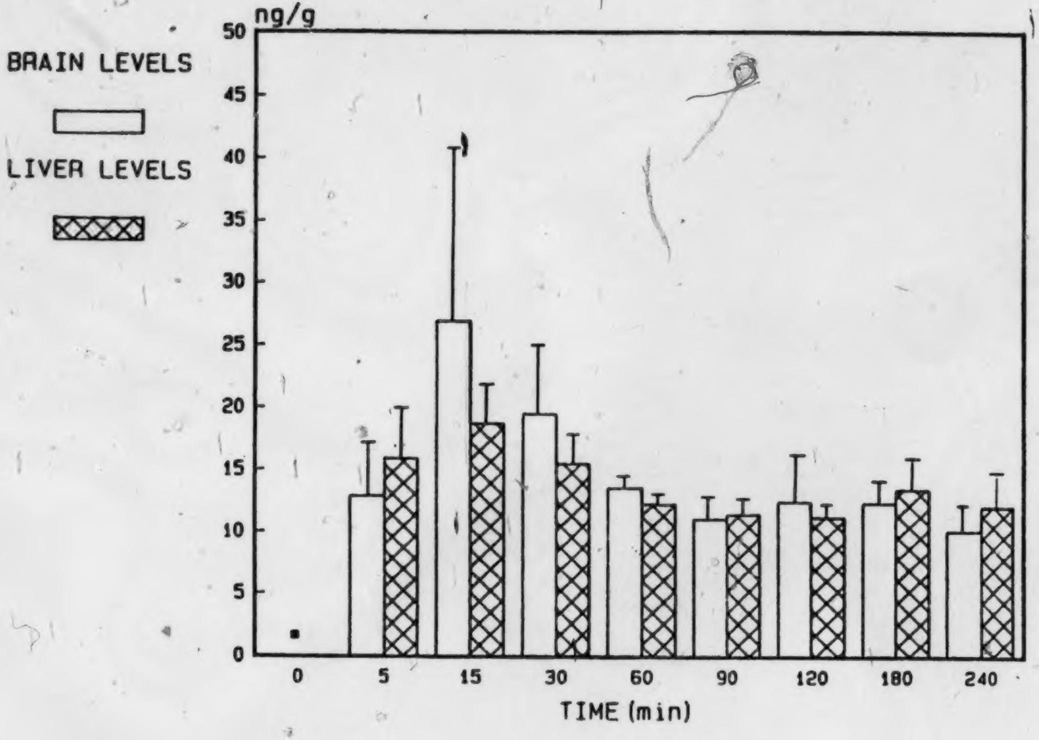


Fig. 46. Rat brain and liver T levels (ng/g, mean ± S.E.M., n=5-6) after administration of PGPEA (0.1 mmol/kg, i.p.).

had been injected with physiological saline at the same time intervals. Following PEA administration, there were significant time-dependent changes in NA levels [F(6,35)=4.19, P<0.005], and levels at 30 and 60 min were significantly below control levels (Fig. 47). There were no significant changes in DA levels [F(6,42)=1.82, P>0.1] (Fig. 48). Administration of PEA resulted in significant time-dependent changes in DOPAC levels [F(6,35)=4.35, P<0.005], with the levels at 15 min being significantly below control levels (Fig. 49). Concentrations of HVA remained unchanged from respective saline controls throughout the 3 hr period (F(6,34)=2.37, P>0.05] (Fig. 50). Statistical analysis indicated no significant time-dependent changes in 5-HT levels following PEA treatment [F(6,39)=1.77, P>0.1]. There were, however, significant time-dependent changes in 5-HIAA levels [F(6,40)=6.1, P<0.001], with levels significantly below control values at 5 and 15 min (Fig. 52).

E.2 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after CEPEA administration

Levels of the neurotransmitter amines and the acid metabolites after CEPEA treatment are illustrated in Figs. 47 to 52 respectively. Following administration of CEPEA there were significant time-dependent changes in NA and DA levels [F(6,44)=2.56, P<0.05; F(6,39)=6.96, P<0.001 respectively]; the levels at 30, 60, 90 and 120 min were significantly below control values in each case. There were no time-dependent changes in DOPAC levels [F(6,53)=1.76, P>0.1], while there were significant time-dependent changes in HVA levels [F(6,51)=3.56, P<0.01]. Levels of HVA were significantly increased above control values at 5, 15 and 30 min. Levels of 5-HT decreased significantly below control values at 30, 60, 90 and 120 min [F(6,51)=3.53, P<0.01]. There were also significant changes in 5-HIAA levels [F(6,46)=2.38, P<0.05]. Multiple mean comparisons did not reveal any significant between-group differences in the case of 5-HIAA. Nevertheless, from the data presented in Fig. 52 it is evident that the overall effect was attributable to increases in 5-HIAA levels at 15, 120 and 180 min.

E.3 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA levels after administration of CPPEA

The levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after administration of CPPEA are shown in Figs. 53 to 55. Concentrations of NA decreased significantly below control levels from 15-180 min after CPPEA treatment [F(6,28)=16.96, P<0.001], and even after 180 min they were approximately 60% of control levels. There were significant time-dependent changes in DA levels [F(6,27)=9.95, P<0.001] and levels at 15, 30, 60 and 90 min were significantly below control values. Statistical analysis indicated significant time-dependent changes in DOPAC levels [F(6,28)=19.41, P<0.001], with increases above control values at 15 min and decreases below control values at 90, 120 and 180 min; the levels at 180 min were approximately 70% of control values. There were significant time-dependent changes in HVA levels [F(6,27)=4.28, P<0.005], and significant time-dependent changes were also observed in 5-HT levels [F(6,28)=9.32, P<0.001]; 5-HT levels increased only at 5 min, and this was followed by a decrease from 15-120 min. There were significant time-dependent changes in 5-HIAA levels [F(6,28)=3.79, P<0.01], with increases above control values being observed at 60 min.

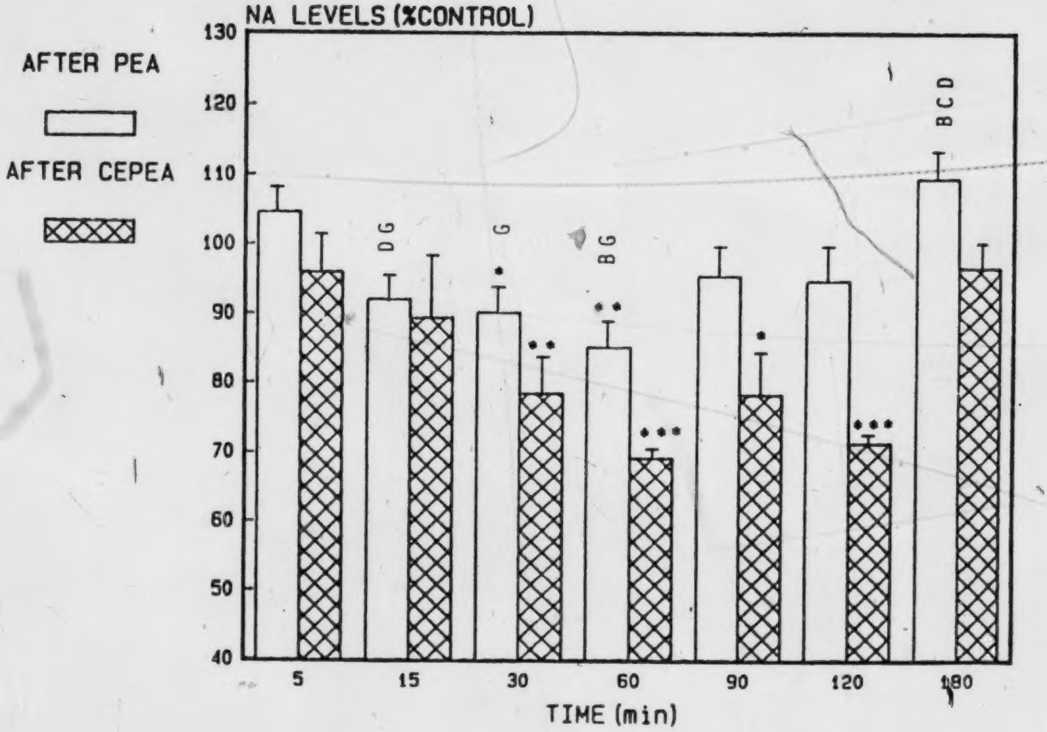


Fig. 47. Rat brain NA levels (% control, mean ± S.E.M., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). (*=P<0.05, **=P<0.001, ***=P<0.001, compared to saline control values at respective times). Control NA levels (pooled from controls sacrificed at different times, ng/g, mean ± S.E.M., n=65) were 328 ± 5.7. Superscripts A, B, C, D, E, F and G refer to significant differences from levels at 5, 15, 30, 60, 90, 120 and 180 min respectively (α=0.05).

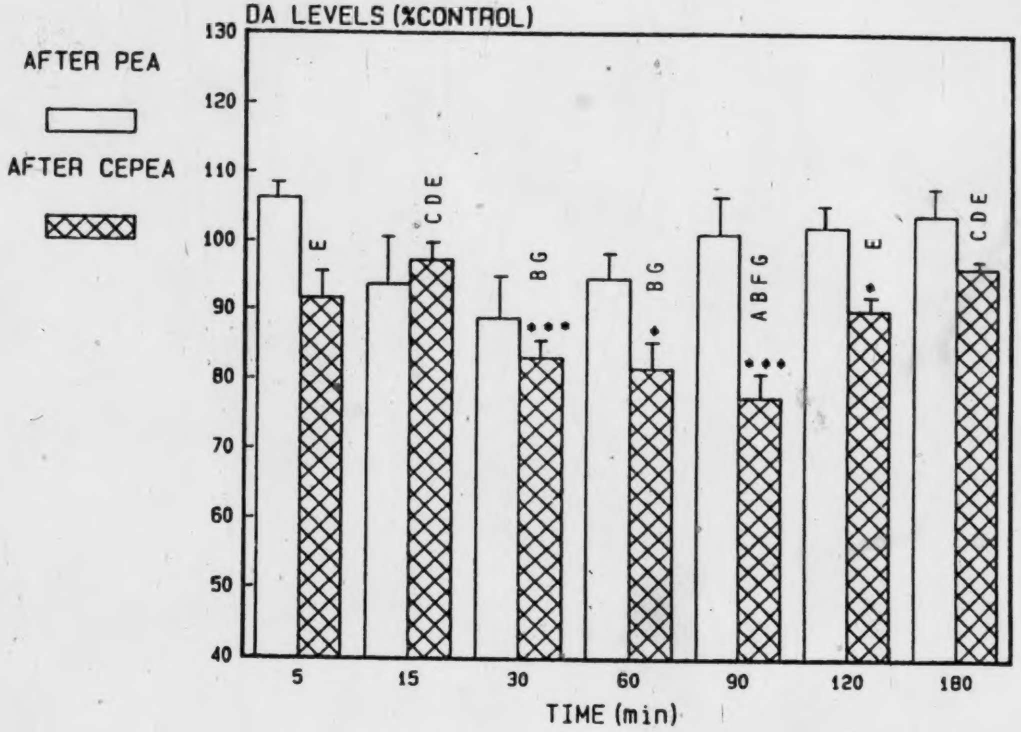


Fig. 48. Rat brain DA levels (% control, mean ± S.E.M., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). Control DA values (ng/g, mean ± S.E.M., n=65) were 675.2 ± 8.3. For statistical notations and superscripts refer to Fig. 47.

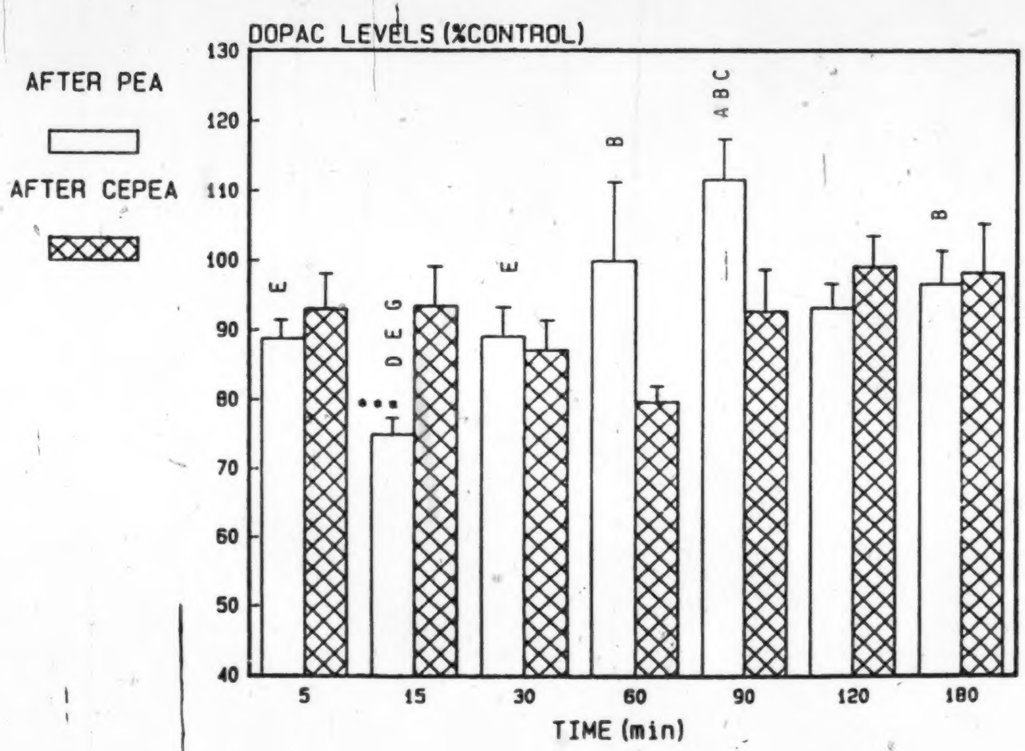


Fig. 49. Rat brain DOPAC levels (% control, mean ± S.E.M., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). Control DOPAC values (ng/g, mean ± S.E.M., n=65) were 109.6 ± 2.7. For statistical notations and superscripts refer to Fig. 47.

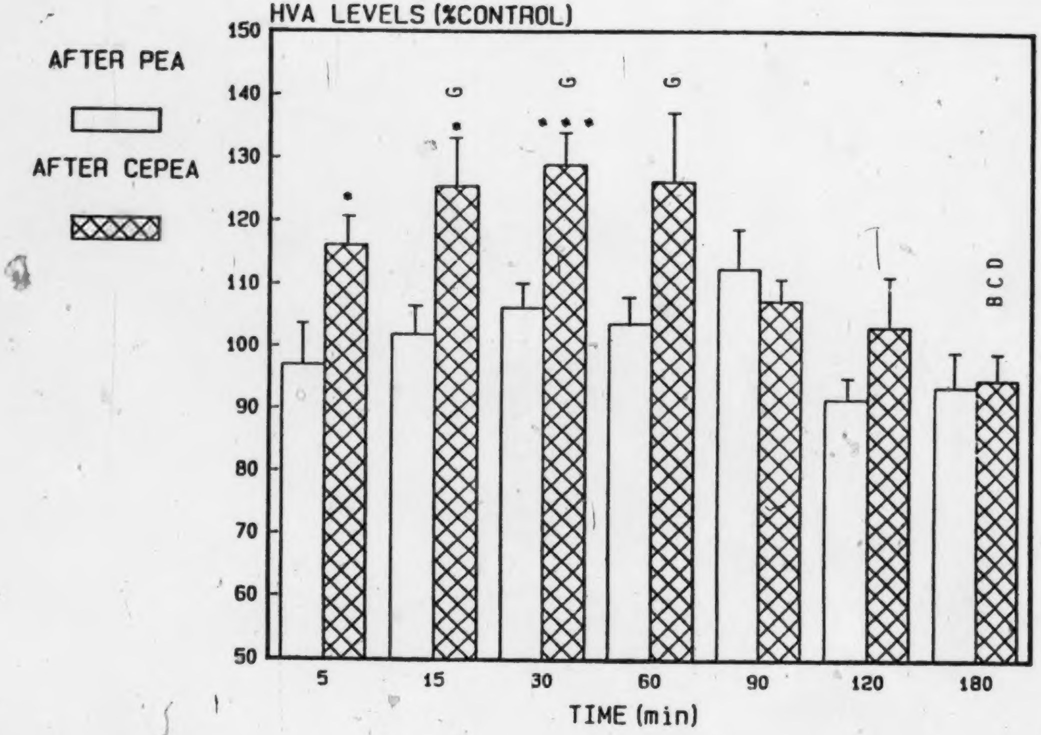


Fig. 50. Rat brain HVA levels (% control, mean ± S.E.M., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). Control HVA values (ng/g, mean ± S.E.M., n=65) were 79.1 ± 1.7. For statistical notations and superscripts refer to Fig. 47.

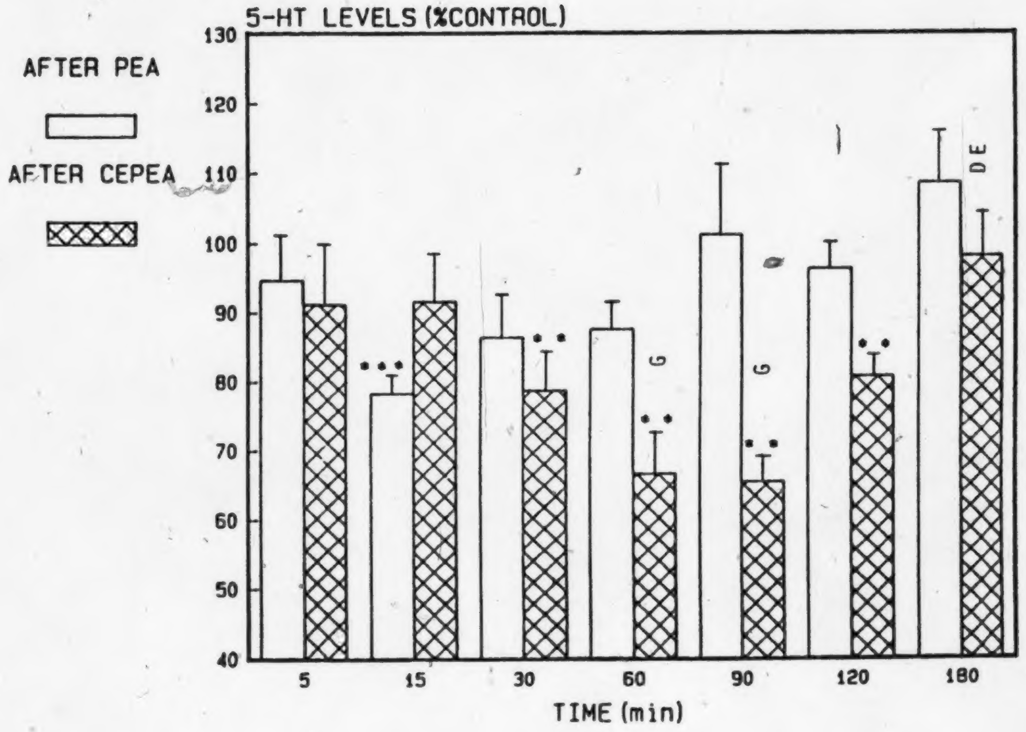


Fig. 51. Rat brain 5-HT levels (% control, mean ± S.E.M.., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). Control 5-HT values (ng/g, mean ± S.E.M., n=65) were 435.0 ± 6.5. For statistical notations and superscripts refer to Fig. 47.

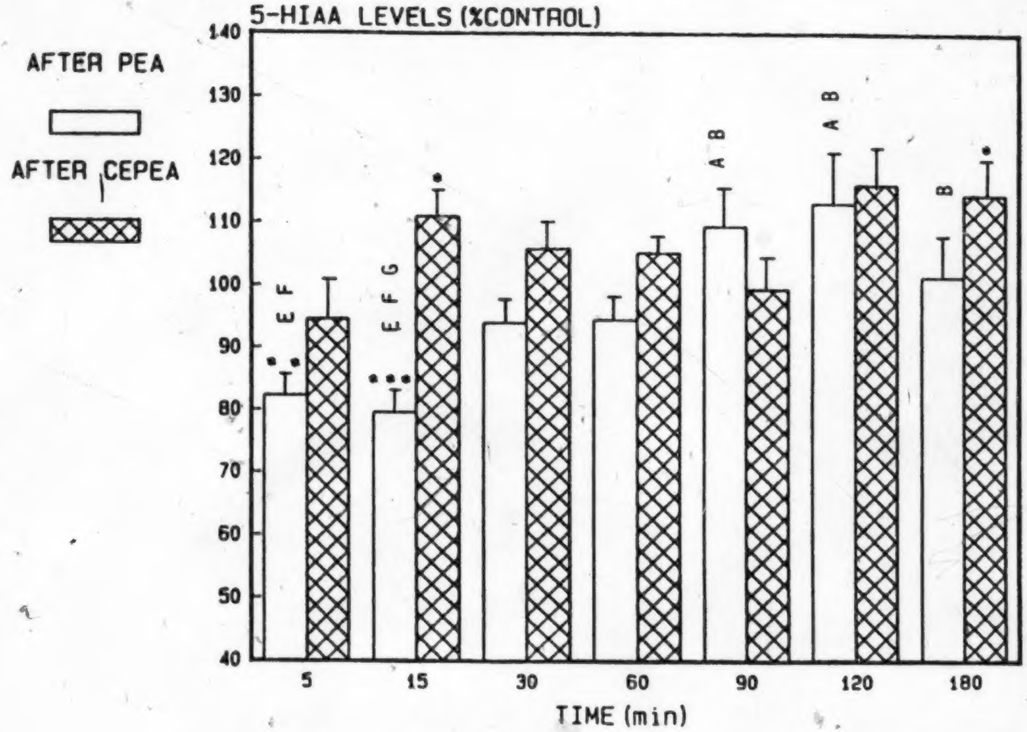


Fig. 52. Rat brain 5-HIAA levels (% control, mean ± S.E.M.., n=5-12) after administration of PEA and CEPEA (0.1 mmol/kg, i.p.). Control 5-HIAA values (ng/g, mean ± S.E.M., n=65) were 286.1 ± 4.3. For statistical notations and superscripts refer to Fig. 47.

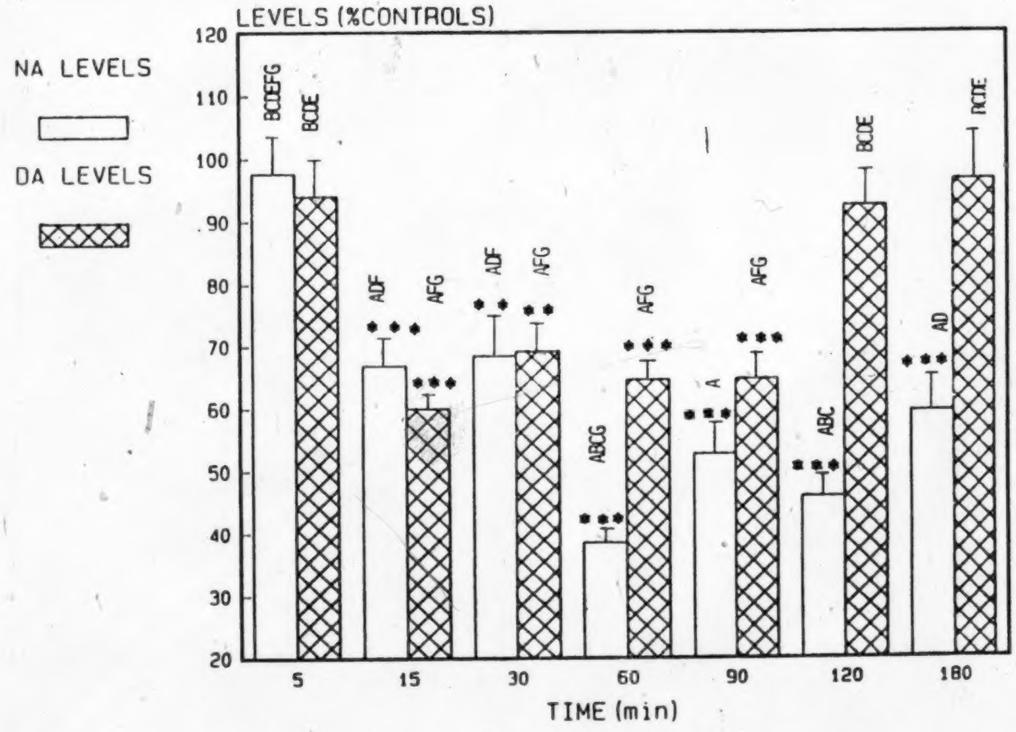


Fig. 53. Rat brain NA and DA levels (% control, mean ± S.E.M., n=5-6) after CPPEA administration (0.1 mmol/kg, i.p.). Control NA and DA levels (pooled from controls sacrificed at different times, ng/g, mean ± S.E.M., n=20) were 331.1 ± 7.0 and 710.2 ± 28.3 respectively. For statistical notations and superscripts refer to Fig. 47.

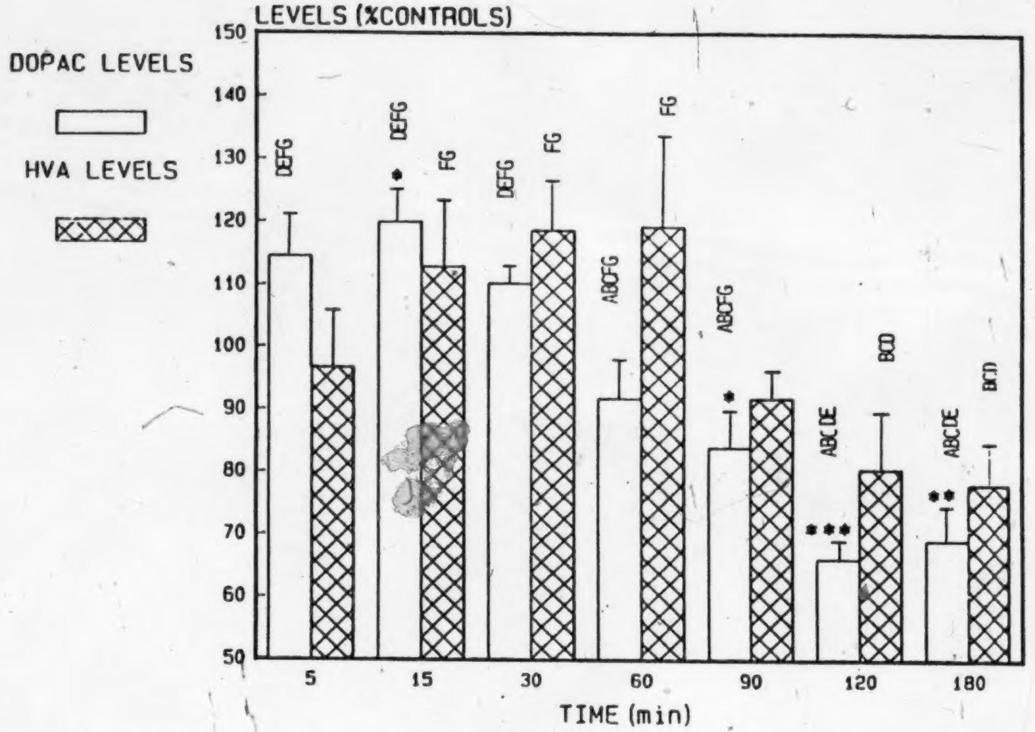


Fig. 54. Rat brain DOPAC and HVA levels (% control, mean ± S.E.M.., n=5-6) after CPPEA administration (0.1 mmol/kg, i.p.). Control DOPAC and HVA levels (ng/g, mean ± S.E.M., n=20) were 104.6 ± 5.2 and 82.5 ± 3.9 respectively. For statistical notations and superscripts refer to Fig. 47.

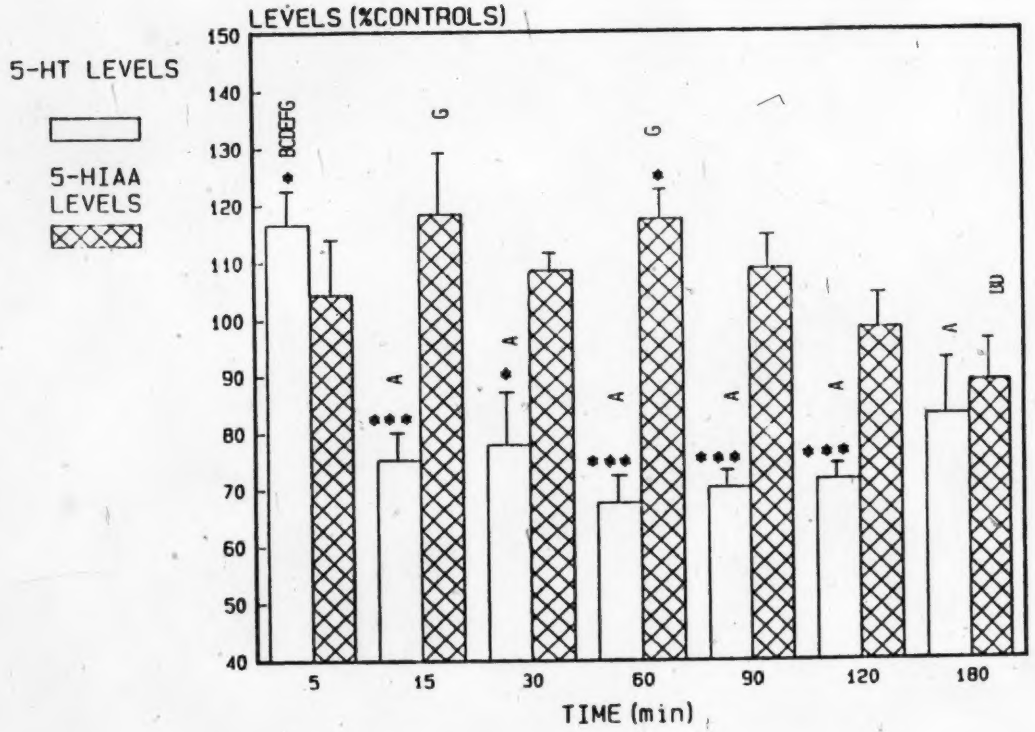


Fig. 55. Rat brain 5-HT and 5-HIAA levels (% control, mean ± S.E.M.., n=5-12) after CPPEA administration (0.1 mmol/kg, i.p.). Control 5-HT and 5-HIAA levels (ng/g, mean ± S.E.M., n=20) were 503.2 ± 31.2 and 287.5 ± 13.2 respectively. For statistical notations and superscripts refer to Fig. 47.

E.4 Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA after the administration of PGPEA

Figures 56 to 61 illustrate the brain NA, DA, DOPAC, HVA, 5-HT and 5-HIAA levels respectively following the administration of PGPEA. There were significant time-dependent changes in NA levels after PGPEA injection [F(6,40)=4.14, P<0.005], and the levels at 5, 30, 60, 90 and 120 min were below control values. Following PGPEA administration, DA levels decreased below control values up to 120 min [F(6,39)=7.19, P<0.001] and by 180 min the levels had increased to control values. There were significant time-dependent changes in DOPAC levels [F(6,49)= 2.34, P<0.05], with levels at 30 and 180 min below control values. Statistical analysis indicated significant time-dependent changes in HVA levels [F(6,38)=12.65, P<0.001]; the levels increased at 5 and 30 min and decreased at 180 min . There were no time-dependent changes in 5-HT levels after PGPEA treatment [F(6,45)=1.13, P>0.1]. However, there were significant time-dependent changes in 5-HIAA levels following PGPEA treatment [F(6,47)=9.03, P<0.001], with levels increased at 5, 15, 120 and 180 min.

E.5 Rat brain levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA following administration of DPGPEA

Figures 62 to 67 illustrate the levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA levels respectively. After DPGPEA treatment there were significant time-dependent changes in NA levels [F(6,39)=3.29, P<0.0025], with decreases below control values at 5, 30, 60, 90 and 120 min. Levels of DA decreased significantly below control values from 5-180 min [F(6,51)=2.34, P<0.05] but multiple mean comparison tests revealed no significant between-group differences (Fig. 63). There were significant time-dependent changes in DOPAC levels [F(7,59)=8.35, P<0.001]; levels from 15 to 180 min were significantly below controls, but multiple mean comparisons indicated no significant between-group differences (Fig. 64). Levels of HVA increased above control values at 5 and 15 min and decreased below control values at 120 and 180 min [F(6,38)=6.92, P<0.001]. No changes in 5-HT levels were apparent [F(6,53)=1.43, P>0.1] during the time-period of the study. There were, however, significant time-dependent changes in 5-HIAA levels [F(6,58)=3.78, P<0.005], and levels increased at 120 and 180 min.

F. Tissue Levels of T and CET

F.1 Rat brain, blood and liver levels of T following the administration of T

The control levels of T in brain, blood and liver could not be quantitated by the present method (aqueous pentafluorobenzoylation, see Section II.D.6), but, based on the sensitivity of the method, they were less than <1 ng/g. Philips *et al.* (1974) reported control levels of 0.5, 0.2 and 0.7 ng/g respectively in these tissues using high resolution mass spectrometry, and these have been assumed to be the control values in the present study.

Semi-logarithmic plots of T levels in brain, blood and liver are presented in Figs. 68, 69 and 70 respectively. There were significant time-dependent changes in T levels in brain, blood and liver [F(5,30)= 45.03, P<0.001; F(5,27)=7.76, P<0.001 and F(6,30)=31.41, P<0.001 respectively], and the multiple mean comparisons are presented in Figs. 68-

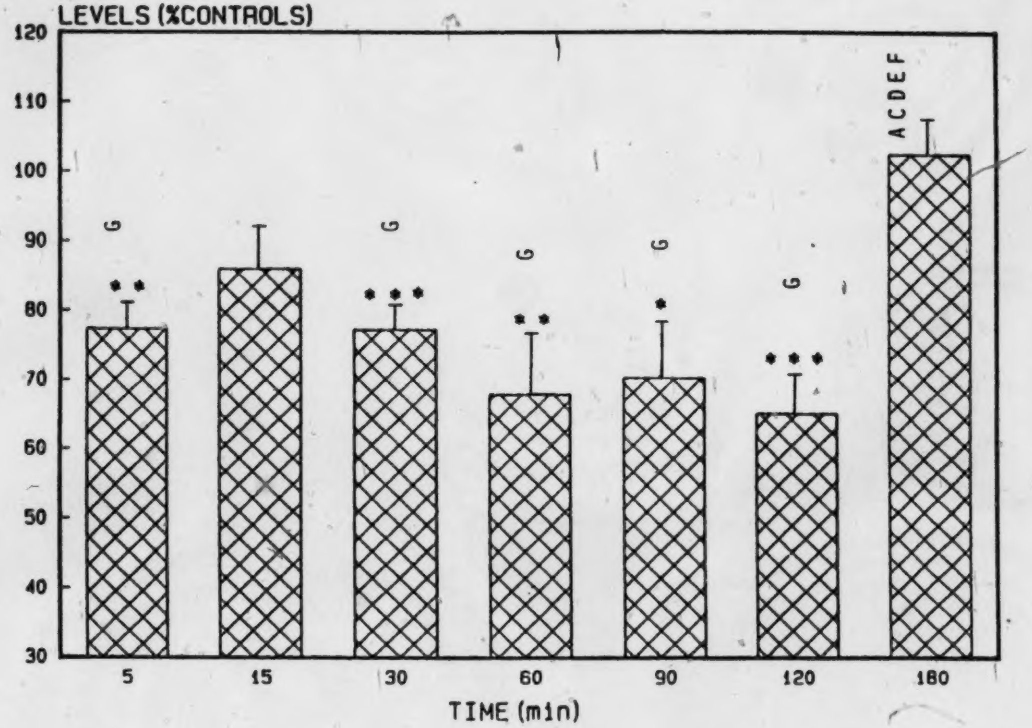


Fig. 56. Rat brain NA levels (% control, mean ± S.E.M., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control NA levels (pooled from controls sacrificed at different times, ng/g, mean ± S.E.M., n=65) were 328.3 ± 5.7. For statistical notations and superscripts refer to Fig. 47.

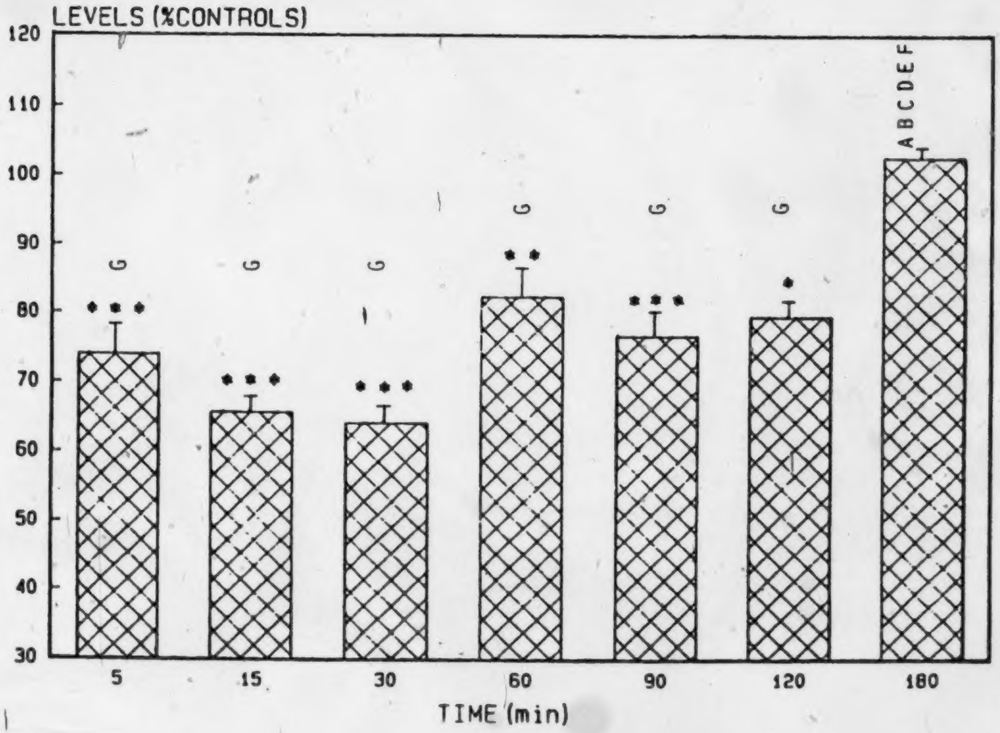


Fig. 57. Rat brain DA levels (% control, mean ± S.E.M.., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control DA levels (ng/g, mean ± S.E.M., n=65) were 675.2 ± 8.3. For statistical notations and superscripts refer to Fig. 47.

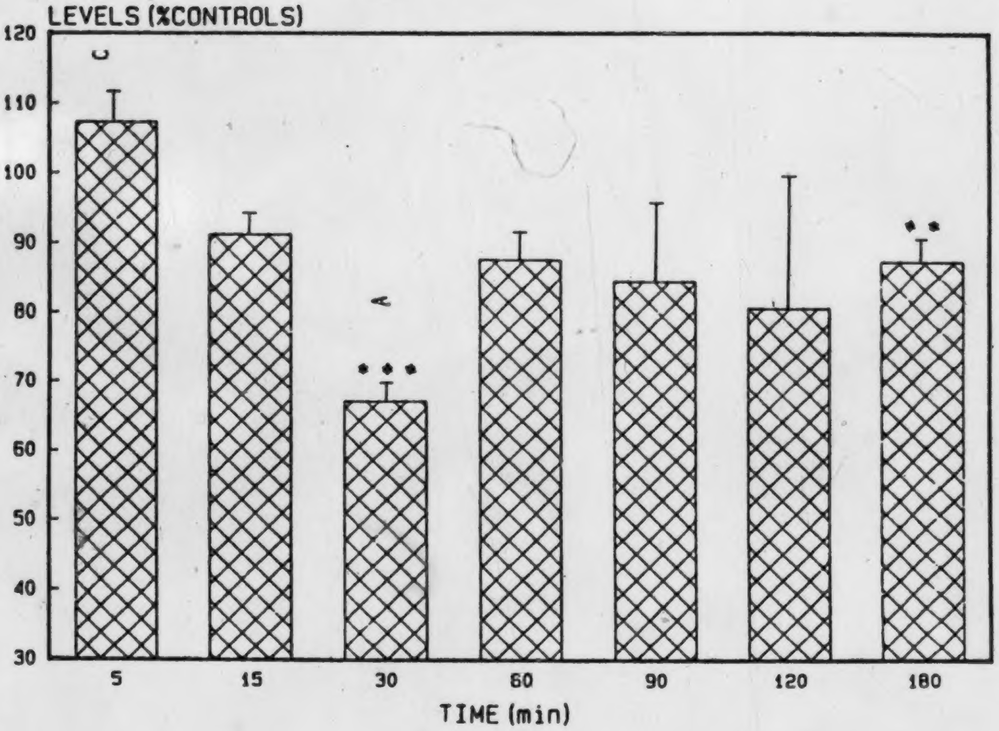


Fig. 58. Rat brain DOPAC levels (% control, mean ± S.E.M.., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control DOPAC levels (ng/g, mean ± S.E.M., n=65) were 109.6 ± 2.7. For statistical notations and superscripts refer to Fig. 47.

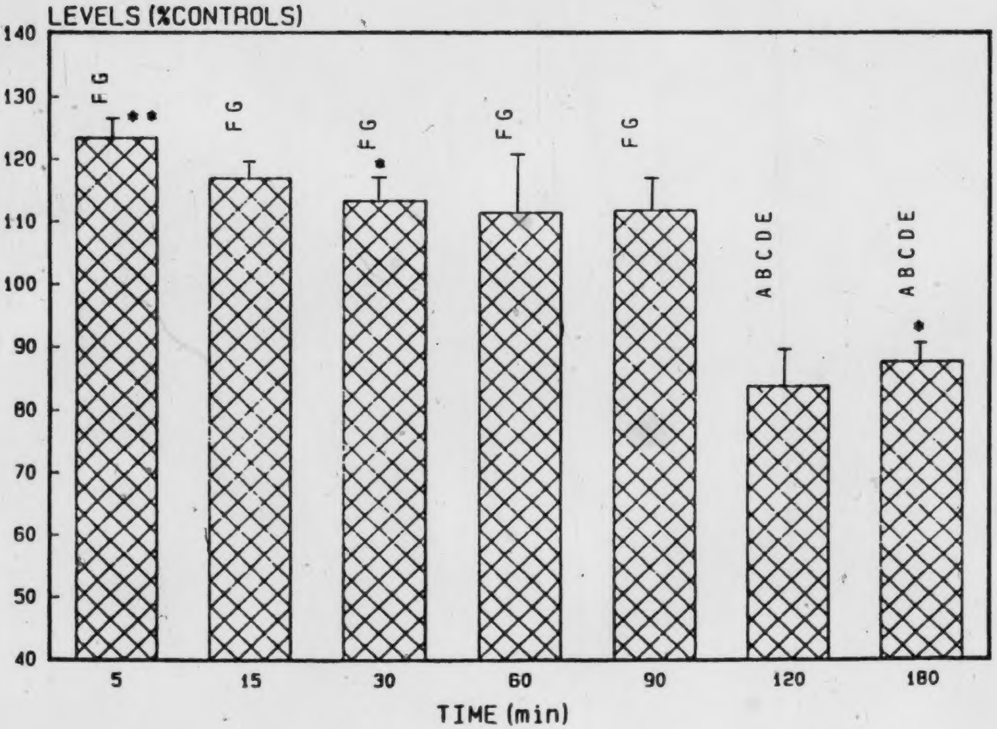


Fig. 59. Rat brain HVA levels (% control, mean ± S.E.M., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control HVA levels (ng/g, mean ± S.E.M., n=65) were 79.1 ± 1.7. For statistical notations and superscripts refer to Fig. 47.

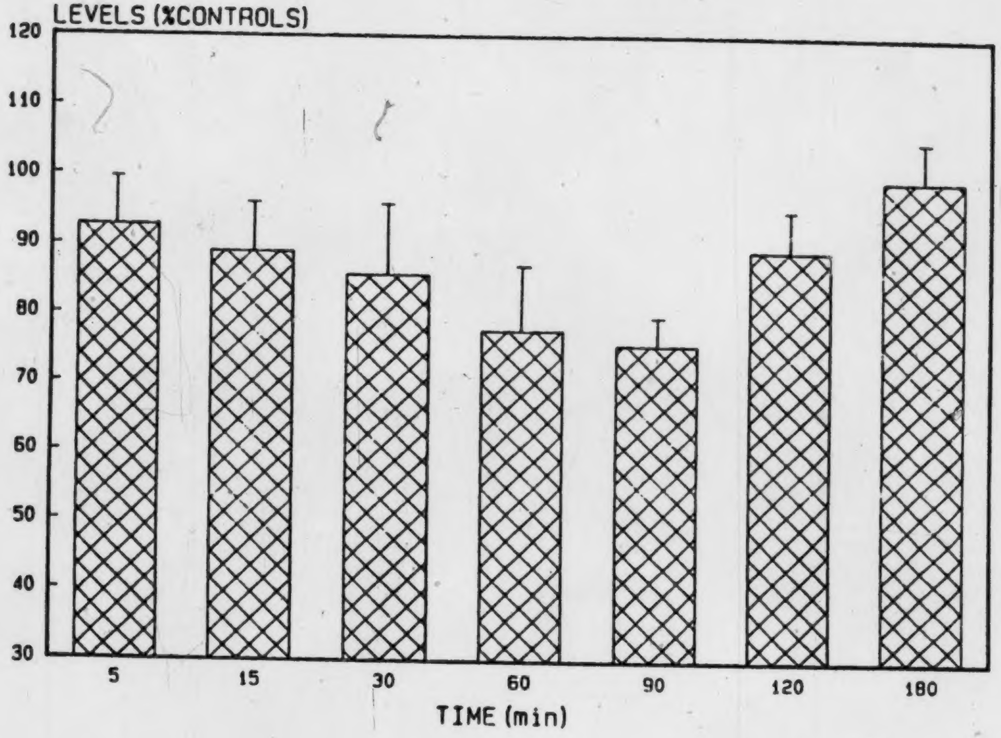


Fig. 60. Rat brain 5-HT levels (% control, mean ± S.E.M.., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control 5-HT values (ng/g, mean ± S.E.M., n=65) were 435.0 ± 6.5. For statistical notations and superscripts refer to Fig. 47.

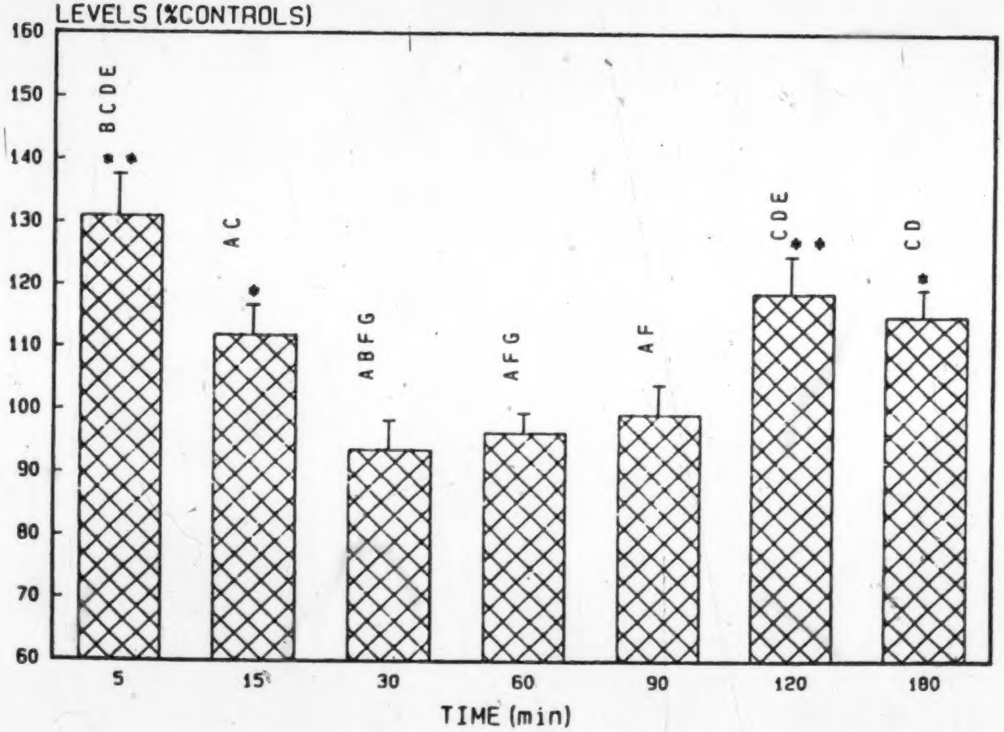


Fig. 61. Rat brain 5-HIAA levels (% control, mean ± S.E.M.., n=5-12) after administration of PGPEA (0.1 mmol/kg, i.p.). Control 5-HIAA levels (ng/g, mean ± S.E.M., n=65) were 286.1± 4.3. For statistical notations and superscripts refer to Fig. 47.

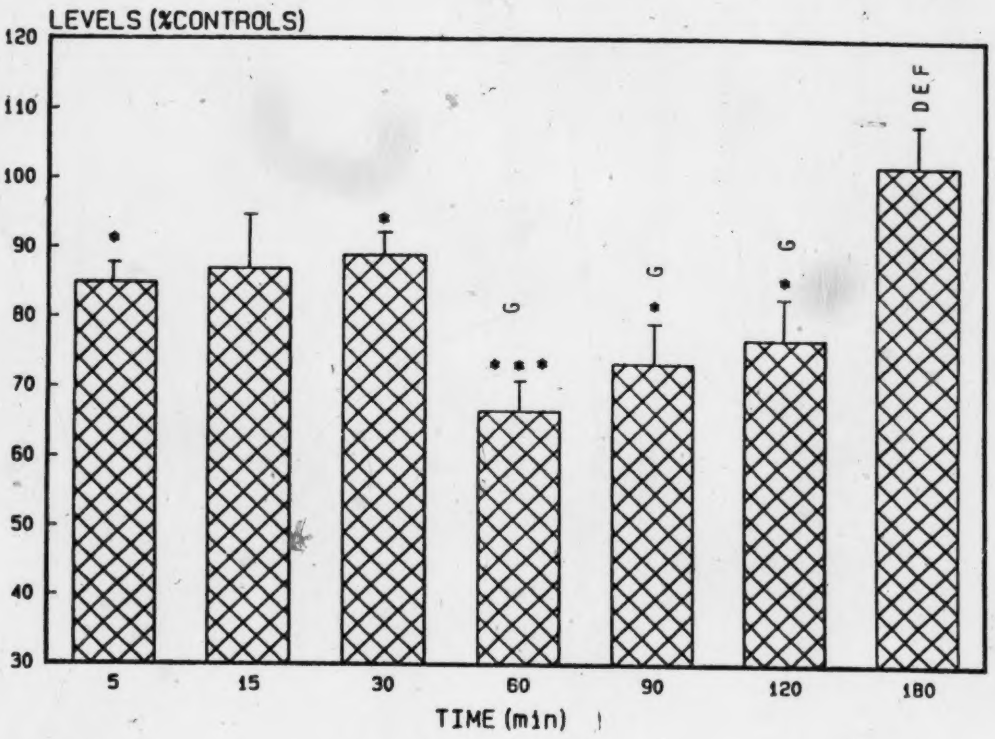


Fig. 62. Rat brain NA levels (% control, mean ± S.E.M.., n=5-12) after administration of DPGPEA (0.1 mmol/kg, i.p.). Control NA levels (ng/g, mean ± S.E.M., n=65) were 328.3 ± 5.7. For statistical notations and superscripts refer to Fig. 47.

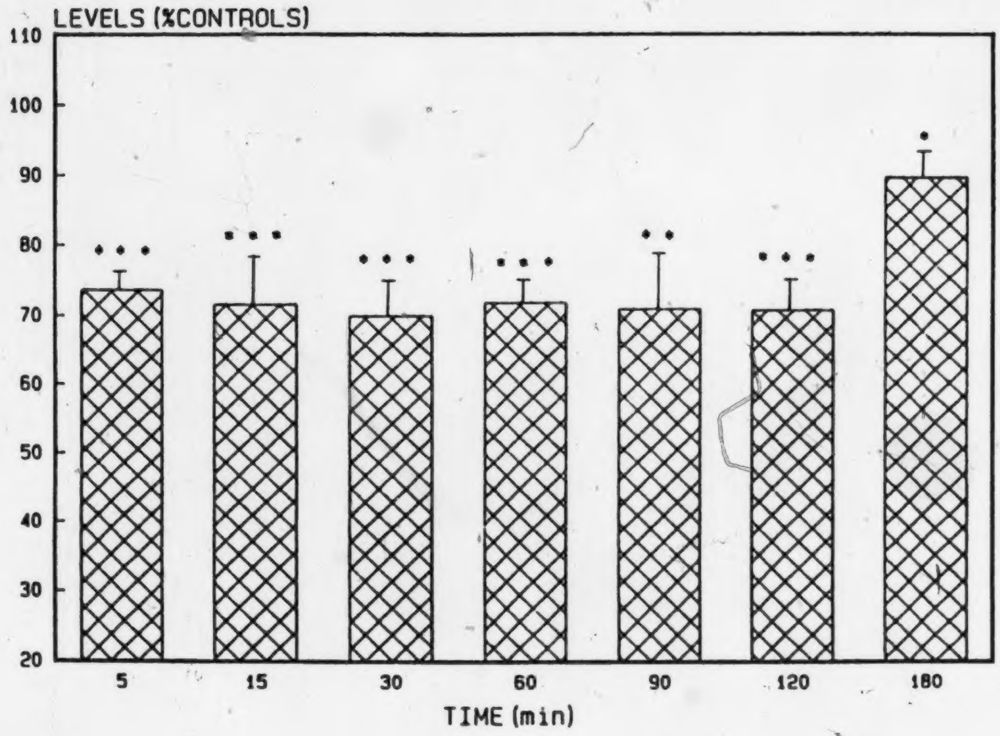


Fig. 63. Rat brain DA levels (% control, mean ± S.E.M., n=5-12) after administration of DPGPEA (0.1 mmol/kg, i.p.). Control DA levels (ng/g, mean ± S.E.M., n=65) were 675.2 ± 8.3. For statistical notations and superscripts refer to Fig. 47.

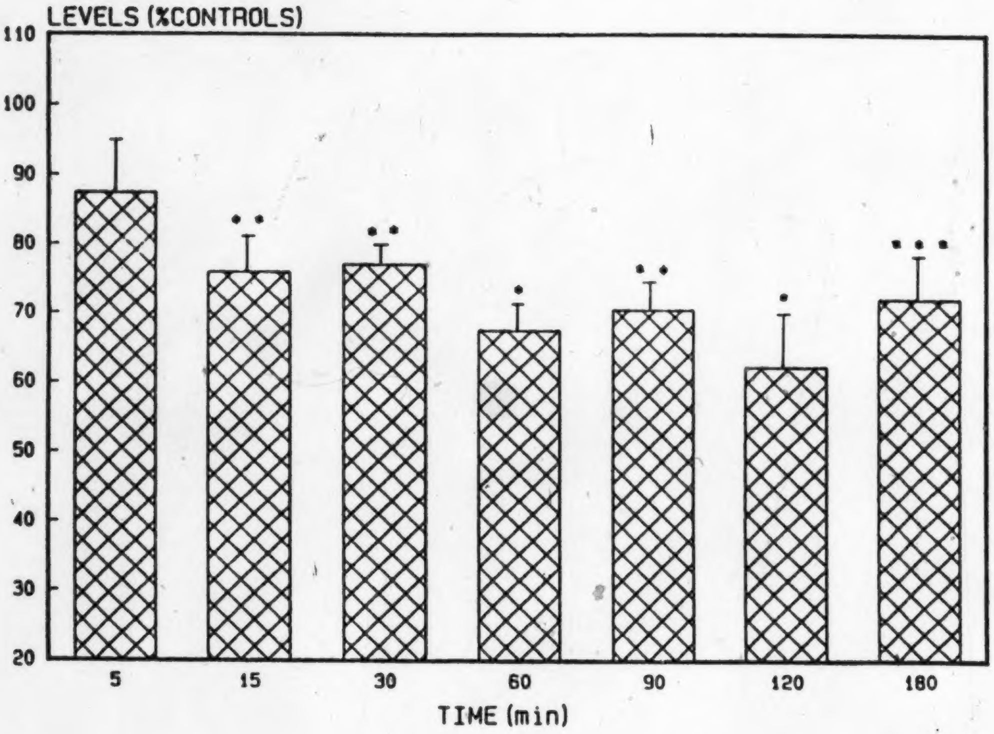


Fig. 64. Rat brain DOPAC levels (% control, mean ± S.E.M.., n=5-12) after administration of DRGPEA (0.1 mmol/kg, i.p.). Control DOPAC levels (ng/g, mean ± S.E.M., n=65) were 109.6 ± 2.7. For statistical notations and superscripts refer to Fig. 47.

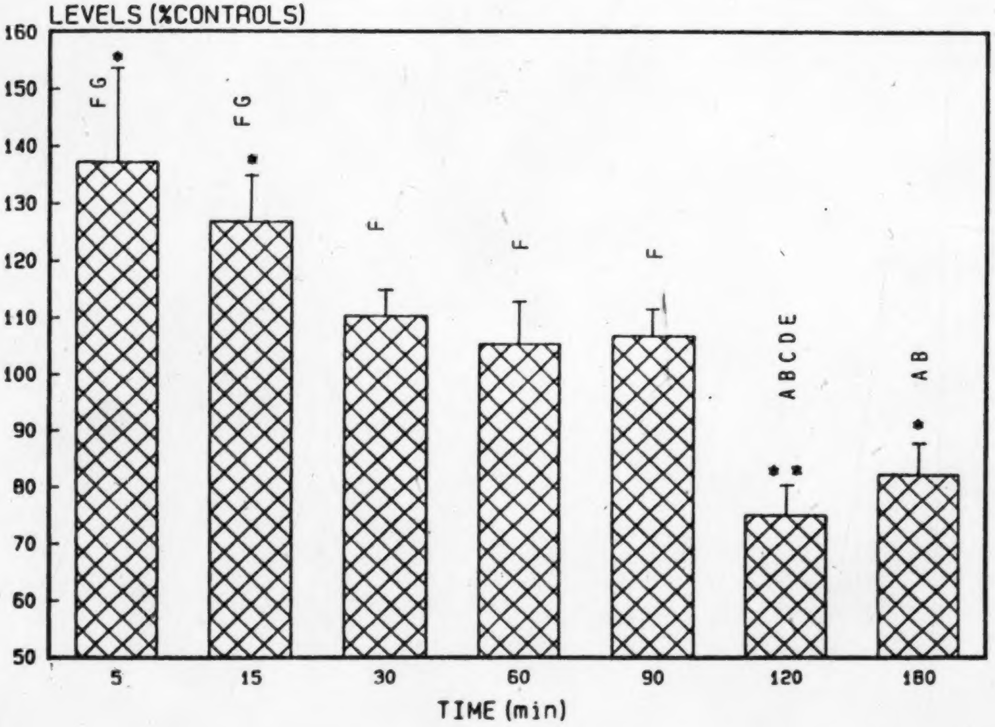


Fig. 65. Rat brain HVA levels (% control, mean ± S.E.M.., n=5-12) after administration of DPGPEA (0.1 mmol/kg, i.p.). Control HVA levels (ng/g, mean ± S.E.M., n=65) were 79.1 ± 1.7. For statistical notations and superscripts refer to Fig. 47.

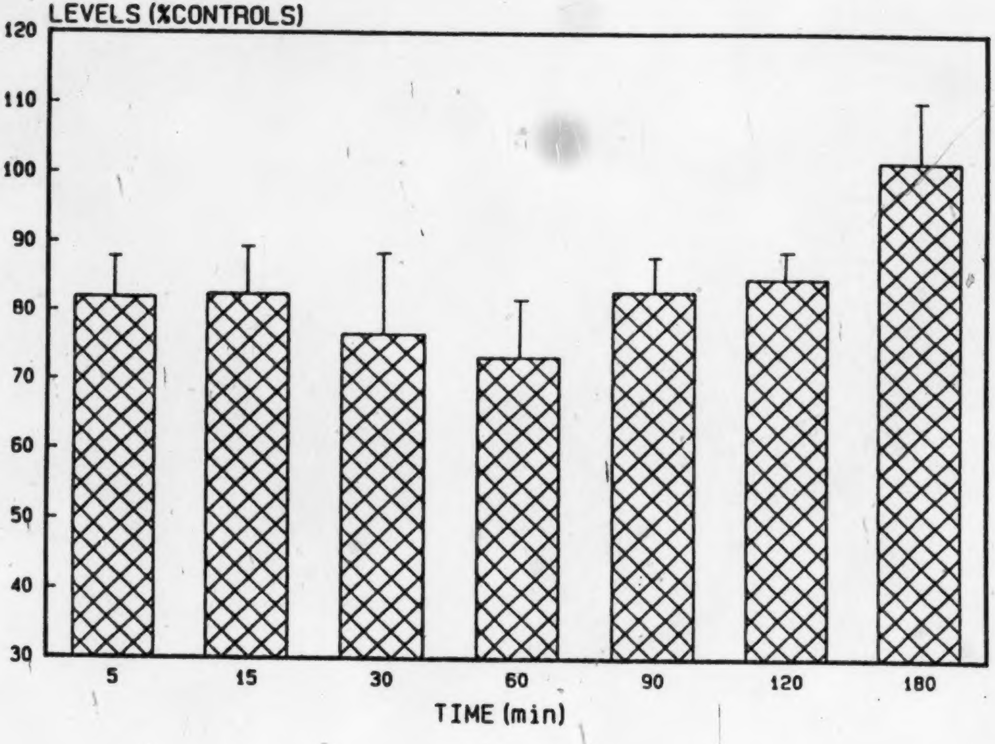


Fig. 66. Rat brain 5-HT levels (% control, mean ± S.E.M.., n=5-12) after administration of DPGPEA (0.1 mmol/kg, i.p.). Control 5-HT values (ng/g, mean ± S.E.M., n=65) were 435.0 ± 6.5. For statistical notations and superscripts refer to Fig. 47.

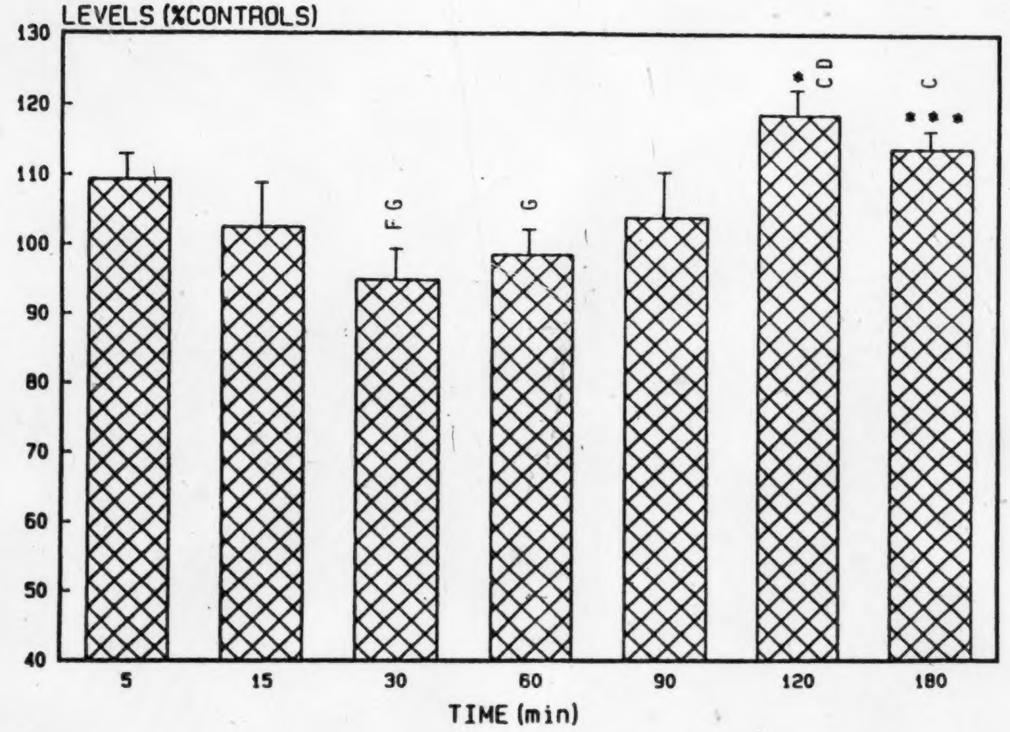


Fig. 67. Rat brain 5-HIAA levels (% control, mean ± S.E.M.., n=5-12) after administration of DPGPEA (0.1 mmol/kg, i.p.). Control 5-HIAA levels (ng/g, mean ± S.E.M., n=65) were 286.1± 4.3. For statistical notations and superscripts refer to Fig. 47.

70. Soon after intraperitoneal administration of T (0.1 mmol/kg) brain levels of T increased significantly above the control levels. Levels at 5 min were nearly 100 times control levels. These levels decreased biexponentially, with a rapid decline followed by a slower elimination. The levels at 120 min were nearly 5 times the control levels and at 180 min they were undetectable (i.e., <1ng/g). In blood, peak levels of nearly 1000 times control values were obtained at 5 min, and these decreased biexponentially to less than 1 ng/g by 180 min. In liver, peak levels of nearly 3200 times control values (assuming control levels of 0.7 ng/g) were obtained at 5 min and decreased biexponentially to about 4 times control levels by 180 min. Peak levels in liver were almost 45 times higher than those in brain (P<0.001) and nearly 10 times higher than those in blood (P<0.001). The rate of elimination from liver was twice as slow as that in brain and blood. The availability of T, as measured by the AUC, in liver was nearly 15 times higher than that in brain (P<0.001) and nearly 7 times higher (P<0.001) than that in blood. The AUC in blood was nearly twice that in brain (P<0.005). The pharmacokinetic parameters are summarized in Table 17.

F.2 Rat brain, blood and liver levels of T and CET following the administration of CET

Semilogarithmic plots of T levels in brain, blood and liver against time are presented in Figs. 68-70. There were significant time-dependent changes in T levels in brain, blood and liver [F(6,35)=6.91, P<0.001; F(6,32)=10.57, P<0.001 and F(6,34)=10.73, P<0.001 respectively] and the multiple mean comparisons are presented in Figs. 68-70. Following the administration of CET (0.1 mmol/kg), T levels in brain, blood and liver increased slowly. In brain, peak levels that were nearly 45 times control levels were obtained at 15 min and they decreased to approximately 10 times control levels by 180 min. The peak levels of T following CET administration were about half of those seen after an equimolar dose of T (P<0.05). In blood, peak levels that were nearly 100 times control values were obtained at 15 min and these decreased to 20 times control levels by 180 min (this was in contrast to levels <1 ng/g by 180 min after T administration). Peak levels of T after CET administration were almost 8 times lower than those seen after an equimolar dose of T (P<0.01). In liver, peak levels that were nearly 250 times control levels were attained at 15 min and decreased to 9 times control levels by 180 min. The peak levels of T after CET injection were about 12 times less than those seen after an equimolar dose of T (P<0.001).

After administration of CET, the availability of T (as measured by the AUC) in liver was nearly 9 times higher than those in brain and blood (P<0.001); there was no difference between AUCs in brain and blood (P>0.2). No difference in the the AUC of T in brain was observed following the injection of either T or CET (P>0.2), while in blood and liver CET administration resulted in significantly lower AUCs (P<0.05 and P<0.02 respectively). In quantitative terms, the decreases in blood and liver were nearly 2 times and 1.7 times respectively. The pharmacokinetic parameters are summarized in Table 17.

Levels of CET in brain, blood and liver are presented in Fig. 71. Peak levels of CET in liver were nearly 3 times and 10 times higher than those in brain (P>0.1) and blood (P<0.05) respectively. The elimination half-lives in these tissues were similar to each other although CET levels could not be detected beyond 120 min in blood whereas they were detectable even at 120 min in brain and liver. The availability of CET as measured by AUC was not different between any two tissues studied ($P>0.05$) although the levels of T formed from it was different in these tissues. The pharmacokinetic parameters are summarized in Table 17.

G. In Vitro and In Vivo Inhibition of MAO by CET

In preliminary *in vitro* studies, inhibitions of MAO-A and -B (using rat brain homogenates as the source of MAO), at a concentration of CET of 1×10^{-5}M were (mean ± S.E.M., n=5-6) 60.2 ± 7.0% and 22.3 ± 4.1% respectively. These data indicate CET to be a somewhat more selective inhibitor of MAO-A than of MAO-B, although in both cases the effect is relatively weak. Studies in brain 60 min following a 0.1 mmol/kg (i.p.) dose of CET showed weak inhibiton of MAO, with values of 9.6 ± 2.4% and 0% (mean ± S.E.M., n=4-6) being obtained for MAO-A and MAO-B respectively. These data indicate rather weak MAO inhibitory activity *in vivo* as well as *in vitro*.

H. Levels of NA, DA, 5-HT, DOPAC, HVA and 5-HIAA Following Administration of T and CET

There were significant time-dependent changes in NA levels after administration of T [F(7,42)=2.63, $P<0.05$] and at 60 min levels had decreased below control values (Fig. 72). Following the administration of CET, NA levels at different times remained unchanged from control values [F(7,44)=0.68, $P>0.2$] (Fig. 72).

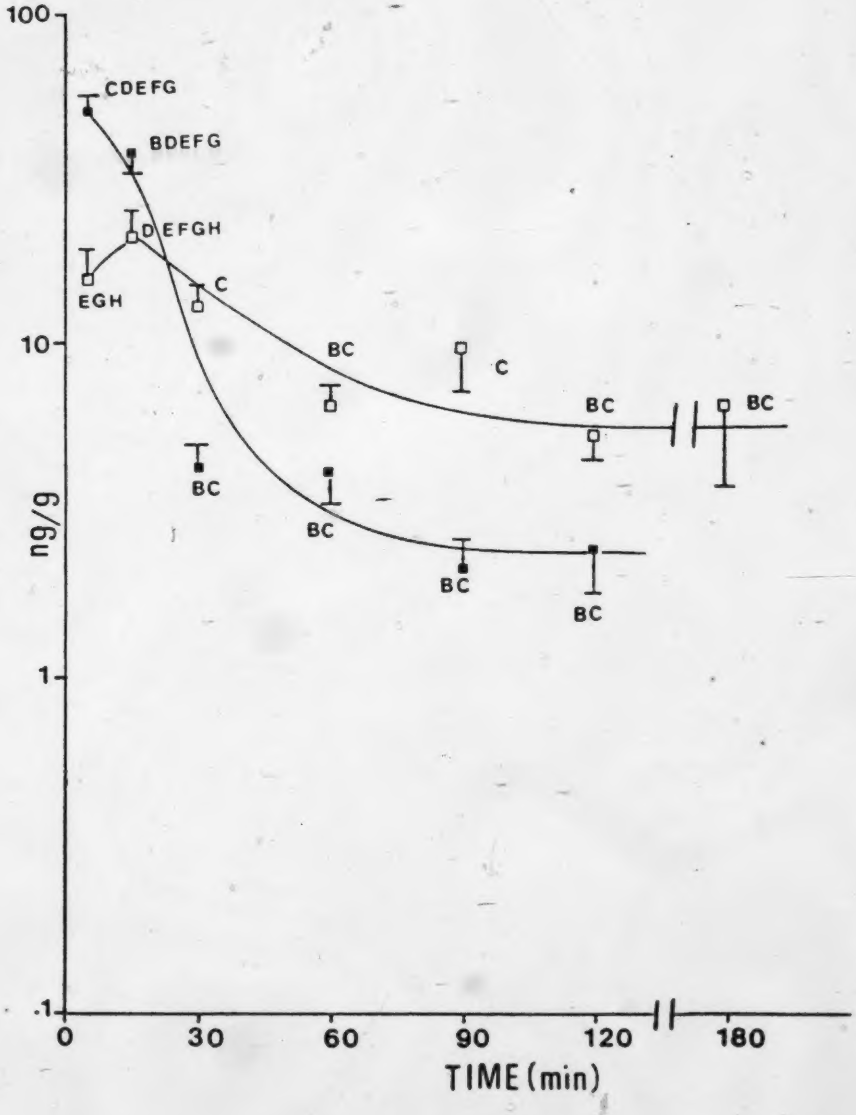


Fig. 68. Rat brain T levels (ng/g, mean ± S.E.M., n=5-6) after administration of T (■) and CET (□) (0.1 mmol/kg, i.p., each). Superscripts B, C, D, E, F, G and H refer to significant differences from levels at 5, 15, 30, 60, 90, 120 and 180 min respectively (α=0.05).

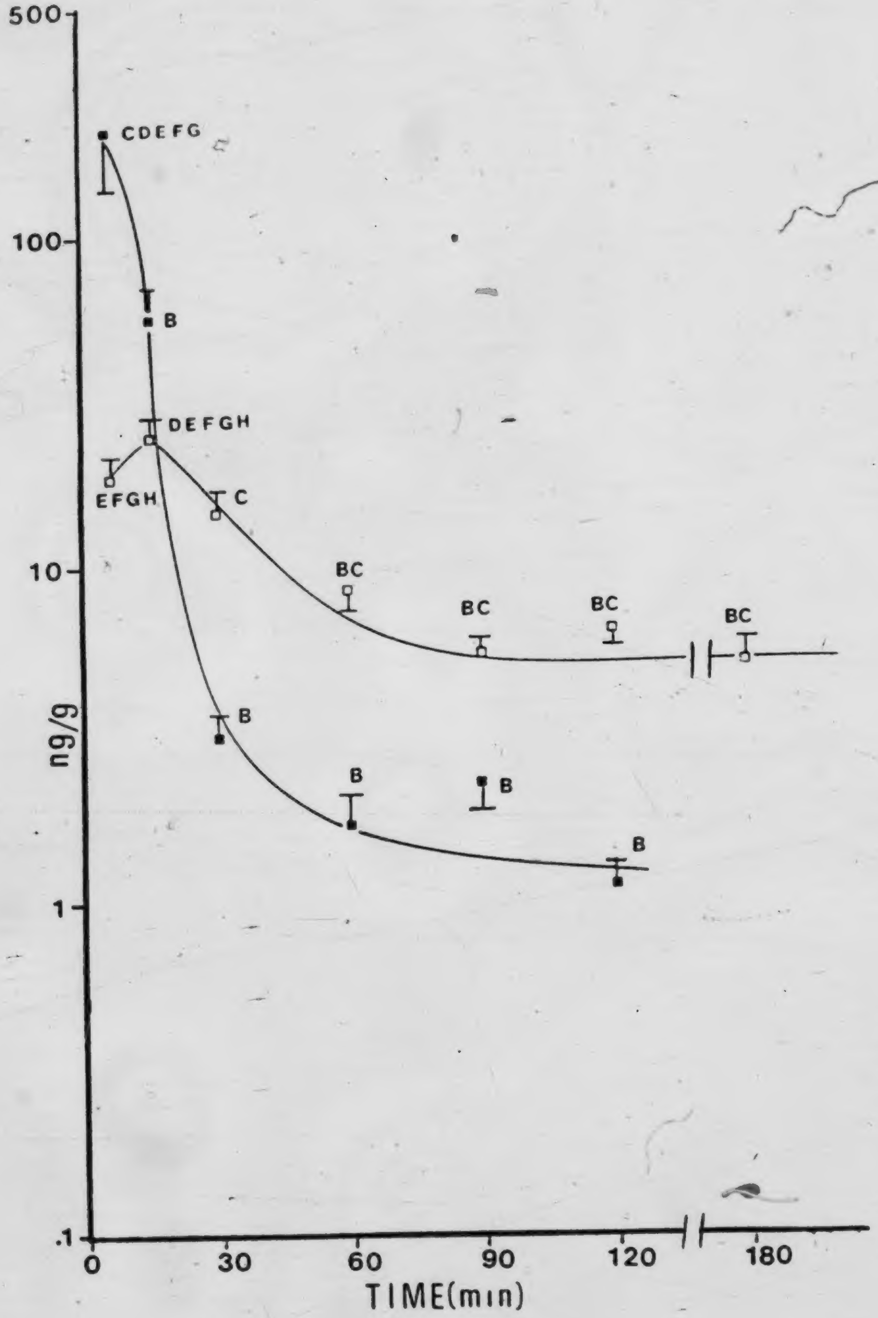


Fig. 69. Rat blood T levels (ng/g, mean ± S.E.M., n=5-6) after administration of T (■) and CET (□) (0.1 mmol/kg, i.p., each). For superscripts refer to Fig. 68.

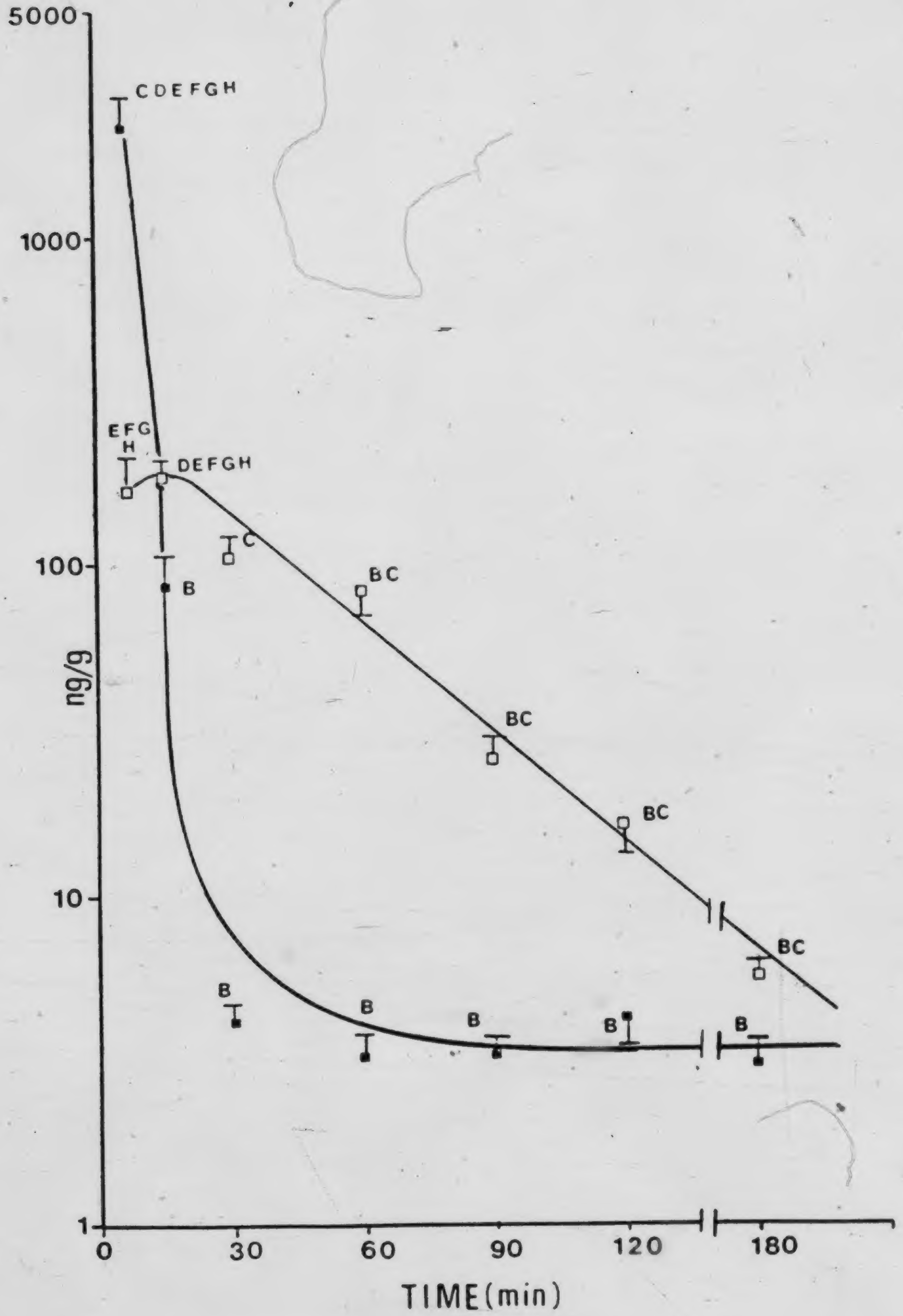


Fig. 70. Rat liver T levels (ng/g, mean ± S.E.M., n=5-6) after administration of T (■) and CET (□) (0.1 mmol/kg, i.p., each). For superscripts refer to Fig. 68.

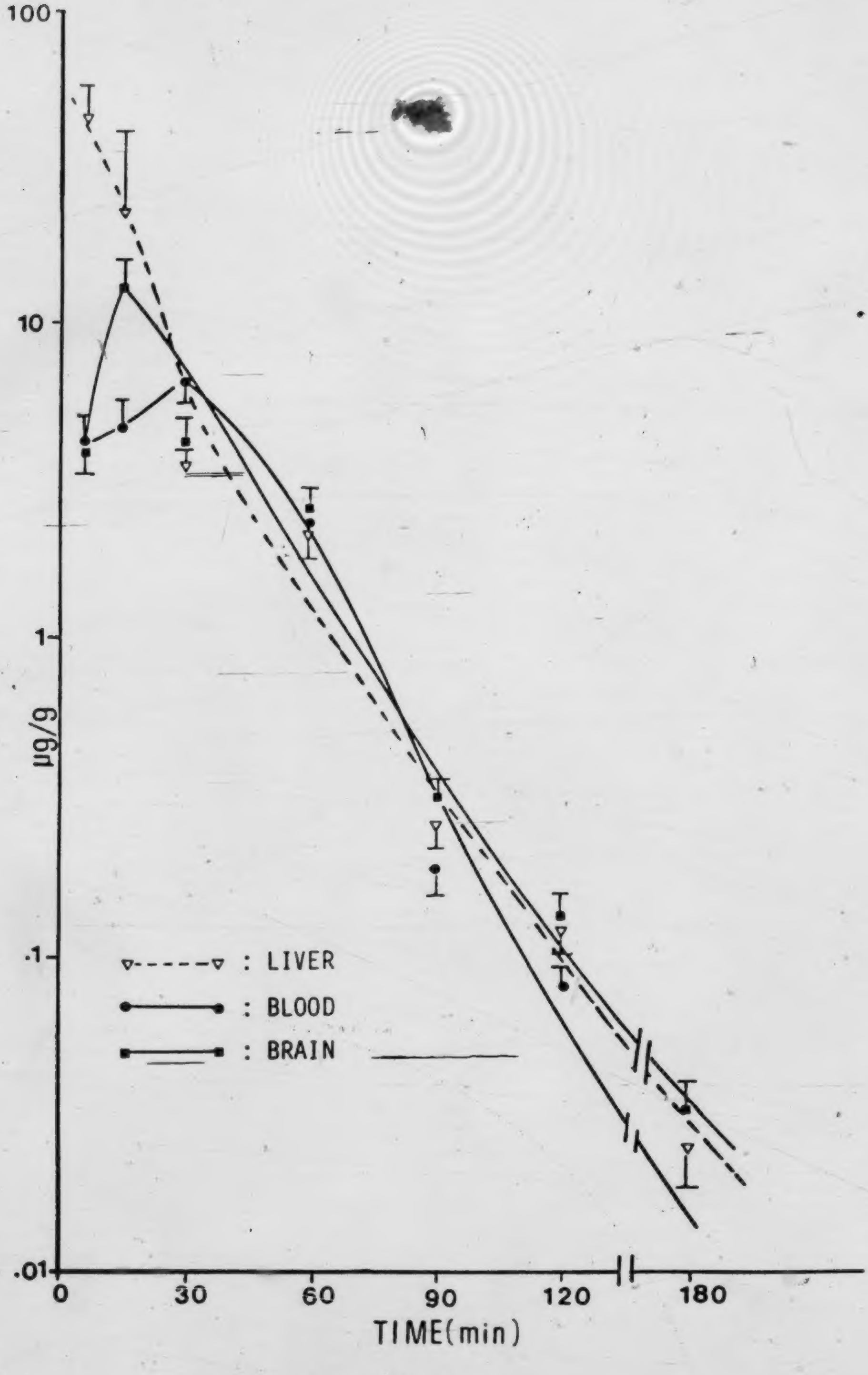


Fig. 71. Rat brain (■), blood (●) and liver (▽) ~~CET~~ CET levels (µg/g, mean ± S.E.M., n=5-6) after administration of CET (0.1 mmol/kg, i.p.).

Table 17. Pharmacokinetic parameters of T and CET in rat tissues following administration of T and CET (0.1 mmol/kg each, i.p.)

	Tissue	AUC^a (nmol/g.min)*	Half-life of Distribution (α) (min)	Half-life of elimination (β) (min)	C_{max}^{a}
T from T	Brain	7.4 ± 0.62	12.5	90**	49.5 ± 7.4^b
	Blood	15.6 ± 2.7	3.5	62	206.9 ± 66.5^b
	Liver	113.9 ± 14.9	3.5	>180	2215 ± 482.4^b
T from CET	Brain	7.8 ± 0.73	-	90	22.8 ± 3.8^b
	Blood	8.1 ± 0.51	14.5	>180	25.1 ± 4.8^b
	Liver	65.8 ± 3.8	-	27	182.8 ± 48.7^b
CET	Brain	1554 ± 474	-	15	13.3 ± 0.93^c
	Blood	1525 ± 184	-	12	6.7 ± 1.7^c
	Liver	3749 ± 1375	-	17.5	44.2 ± 15.6^c

^{a}=mean ± S.E.M., n=5-6
*=0-120 min
**=based on brain levels up to 120 min
^{b}=ng/g
^{c}= μg/g

There were significant time-dependent changes in DA levels following T administration over the time period of study [F(7,42)=14.08, P<0.001], and levels were significantly below control values at 5, 30, 60, 90, 120 and 180 min (Fig. 73). Following CET administration there were significant time-dependent changes in DA levels [F(7,44)=5.53, P<0.001] (Fig. 73); levels increased above controls up to 30 min and decreased thereafter, but none of these subsequent changes were significantly different from control values.

Following the administration of T there were significant time-dependent changes in DOPAC levels [F(7,40)=7.86, P<0.001], with significant decreases from 5 to 120 min; by 180 min levels had returned to control levels (Fig. 74). Following CET administration there were also significant time-dependent differences in DOPAC levels [F(7,44)=19.23, P<0.001], with significant increases from 5-60 min and a return thereafter to control values (Fig. 74).

Administration of T resulted in significant time-dependent changes in HVA levels [F(7,42)=10.76, P<0.001]; they were increased significantly above controls at 5 and 15 min and decreased thereafter. At 120 min HVA levels were significantly below control values and by 180 min the trend to increase to control levels was clearly evident. Multiple mean comparisons are presented in Fig. 75. Following CET administration, HVA levels remained unchanged from control values throughout the time period of study [F(7,44)=3.54, P<0.01] (Fig. 75).

There were significant time-dependent changes in 5-HT levels following T administration [F(7,41)=3.36, P<0.01] (Fig. 76), and levels at 60 min were below control levels. Significant time-dependent changes in 5-HT levels were also evident after CET administration [F(7,43)=6.04,

$P<0.001$]; in this case 5-HT levels at 5 min were higher than control values (Fig. 76).

Concentrations of 5-HIAA remained unchanged from control values from 5-180 min after administration of T [$F(7,43)=1.12$, $P>0.1$]. There were, however, significant time-dependent changes in 5-HIAA levels after the administration of CET [$F(7,44)=17.68$, $P<0.001$], with levels decreasing significantly below controls at 5, 15 and 30 min and increasing significantly above control values at 180 min (Fig. 77).

I. In Vitro MAO Inhibitory Activities of Structural Analogs of TCP

Several structural analogs of TCP were examined for their abilities to inhibit rat brain MAO-A and -B *in vitro* by employing specific substrates for these two enzymes. The results are tabulated in Table 18. From the data it is evident that (+)-TCP was more potent at inhibiting MAO-A than TCP and (-)-TCP, and (+)-TCP was more potent than (-)-TCP at inhibiting MAO-B. Among the analogs, FTCP was nearly 10 times more potent than TCP against MAO-A and -B and 2-naphthylcyclopropylamine (NCP) was nearly 10 times more potent than TCP at inhibiting MAO-A. Compounds such as 3,4-methylenedioxytranylcypromine (MDTCP), 3-trifluoromethyltranylcypromine (TTCP), and 4-methoxytranylcypromine (MTCP) were equipotent to TCP against MAO-A and -B. N-Acylation or N-alkylation in general decreased the potency, although the degree of this effect was variable depending upon the nature of the substituent.

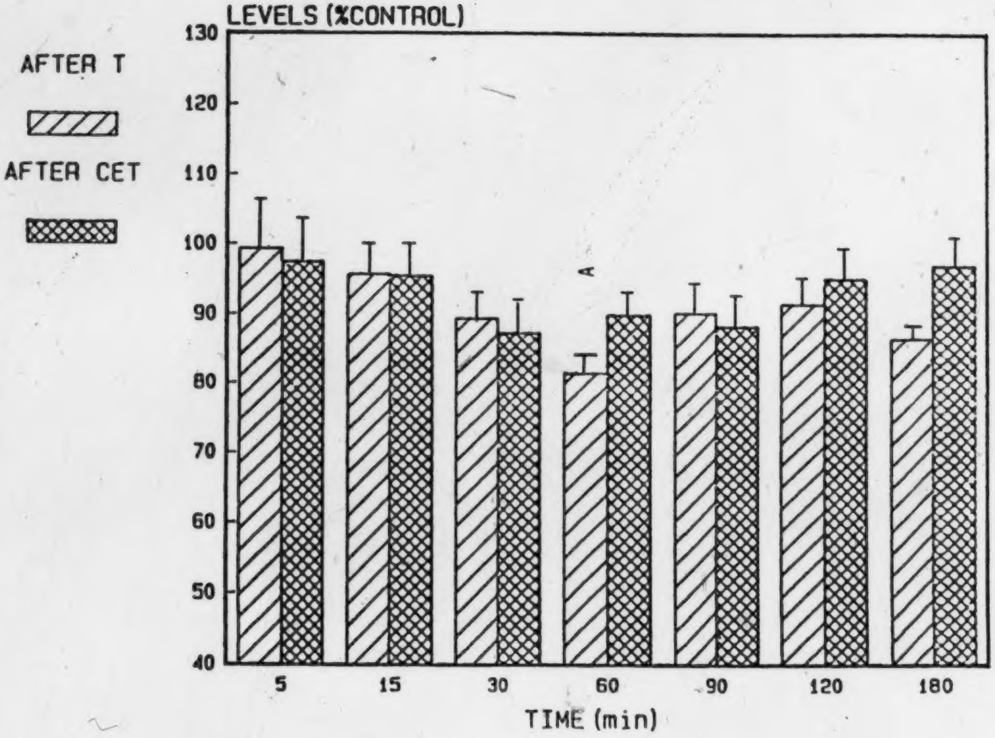


Fig. 72. Rat brain NA levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). (*=P<0.05, **=P<0.01 and ***=P<0.001 indicate differences between T- and CET-treated groups). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 0, 5, 15, 30, 60, 90, 120 and 180 min respectively (α=0.05). Control NA levels (ng/g, mean ± S.E.M., n=10) were 341.2 ± 11.82.

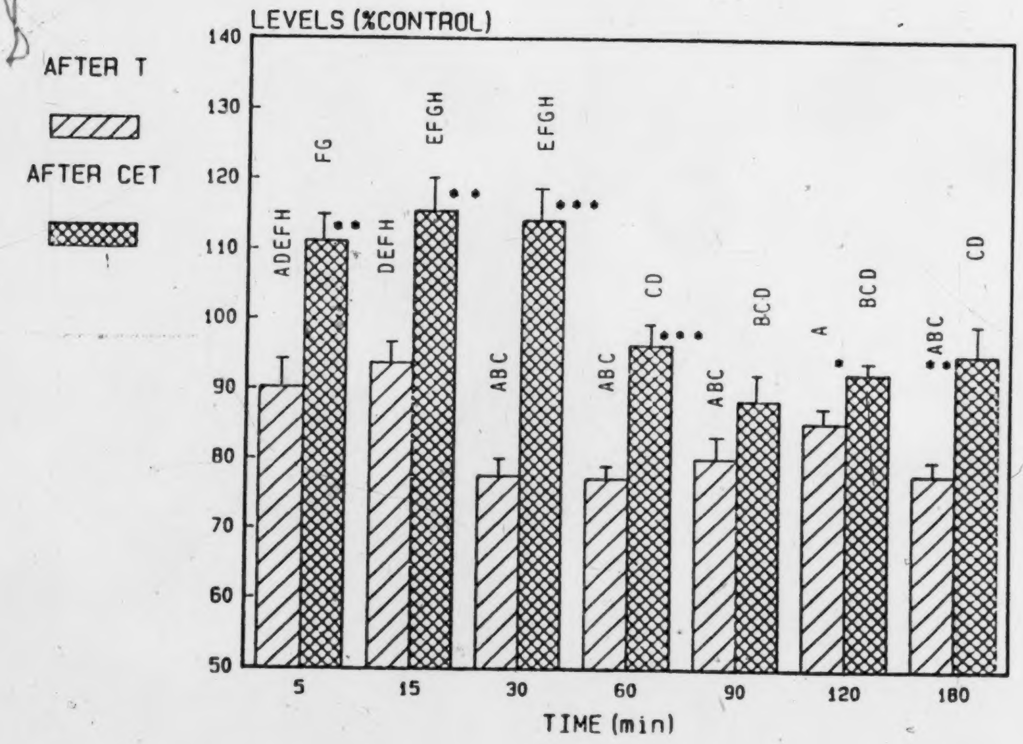


Fig. 73. Rat brain DA levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). Control DA levels (ng/g, mean ± S.E.M., n=10) were 680.7 ± 14.7. For statistical notations and superscripts refer to Fig. 72.

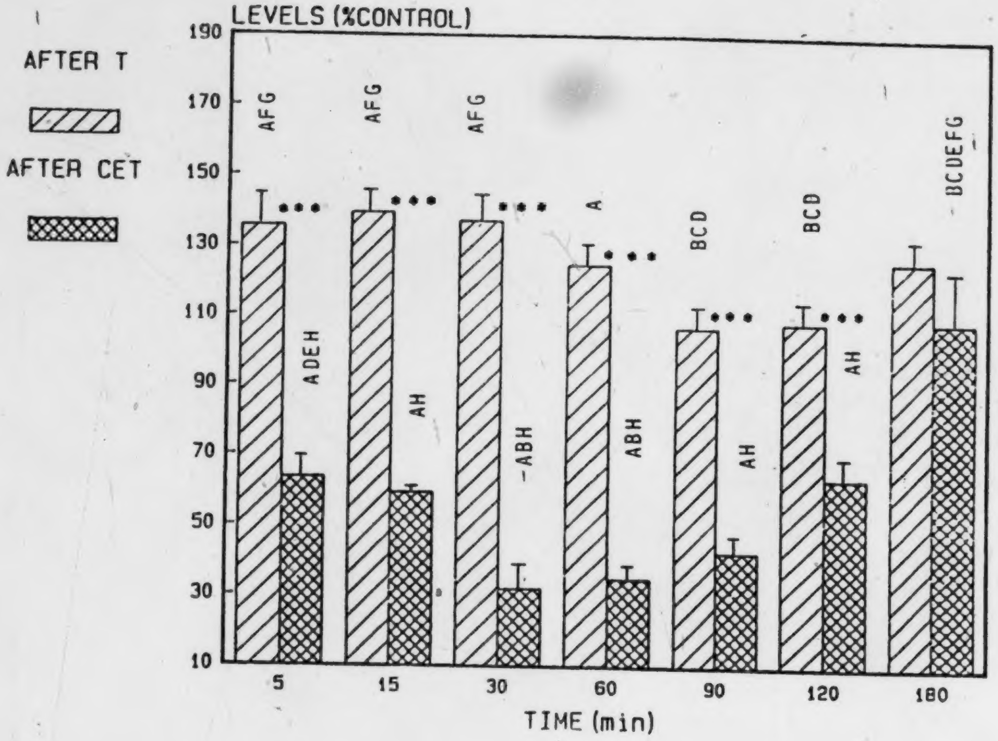


Fig. 74. Rat brain DOPAC levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). Control DOPAC levels (ng/g, mean ± S.E.M., n=10) were 99.6 ± 3.3. For statistical notations and superscripts refer to Fig. 72.

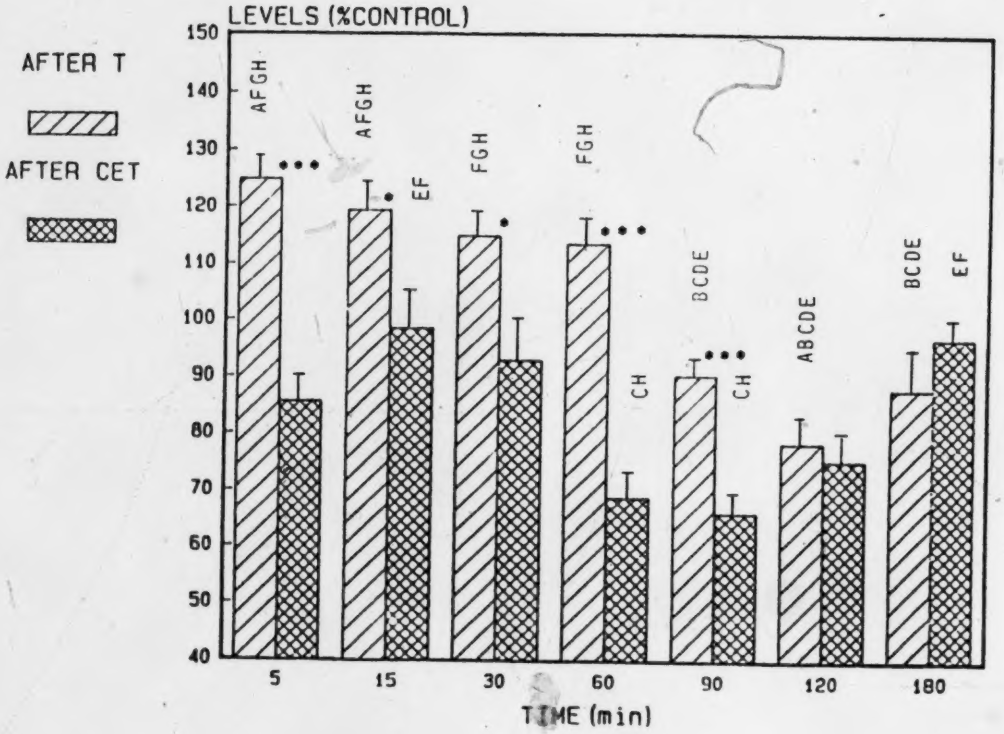


Fig. 75. Rat brain HVA levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). Control HVA levels (ng/g, mean ± S.E.M., n=10) were 87.3 ± 4.5. For statistical notations and superscripts refer to Fig. 72.

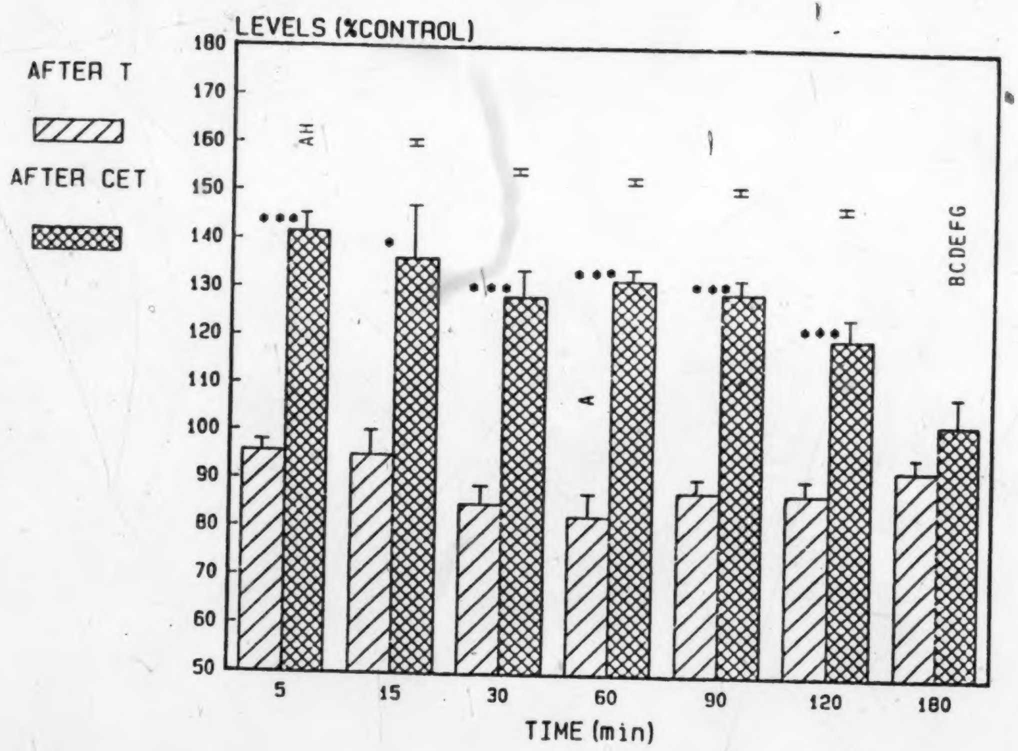


Fig. 76. Rat brain 5-HT levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). Control 5-HT levels (ng/g, mean ± S.E.M., n=10) were 427.3 ± 12.6. For statistical notations and superscripts refer to Fig. 72.

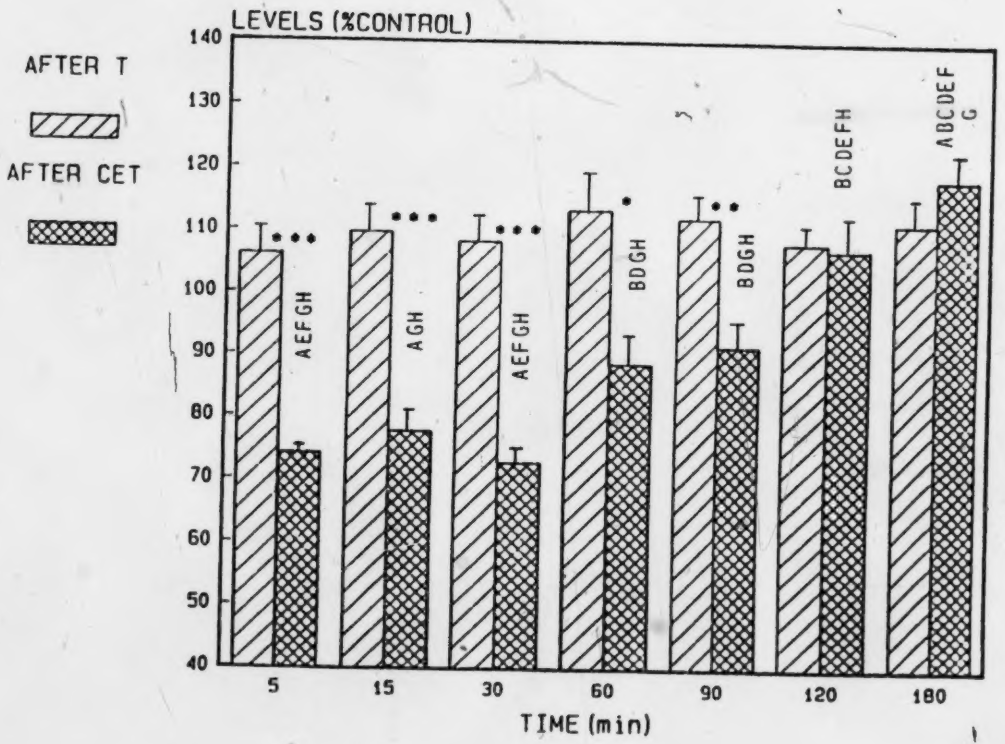


Fig. 77. Rat brain 5-HIAA levels (% control, mean ± S.E.M., n=5-6) after administration of T and CET (0.1 mmol/kg, i.p.). Control 5-HIAA levels (ng/g, mean ± S.E.M., n=10) were 309.5 ± 15.1. For statistical notations and superscripts refer to Fig. 72.

Table 18. Effects of TCP and some of its analogues on inhibiton of MAO in rat brain *in vitro*.

Drug	IC_{50} (M) MAO-A	IC_{50} (M) MAO-B
(±)-TCP	7.4×10^{-8}	9.0×10^{-8}
(+)-TCP	7.7×10^{-9}	6.7×10^{-8}
(-)-TCP	9.5×10^{-7}	5.9×10^{-6}
FTCP	9.1×10^{-9}	9.5×10^{-9}
N-(2-Cyanoethyl)-tranylcypromine (CETCP)	5.8×10^{-7}	9.0×10^{-7}
3-Trifluoromethyltranylcypromine (TTCP)	8.4×10^{-8}	7.9×10^{-8}
4-Methoxytranylcypromine (MTCP)	4.8×10^{-8}	7.1×10^{-8}
2-Napthylcyclopropylamine (NCP)	9.8×10^{-9}	6.7×10^{-8}
3,4-Methylenedioxytranylcypromine (MDTCP)	8.6×10^{-8}	9.2×10^{-8}
N-Acetyltranylcypromine (ATCP)	7.2×10^{-5}	1.6×10^{-4}
N-Ethoxycarbonyltranylcypromine (ECTCP)	8.7×10^{-5}	1.1×10^{-3}
N-Isobutoxycarbonyltranylcypromine (ICTCP)	9.6×10^{-5}	8.5×10^{-4}
N-Propargyltranylcypromine (PGTCP)	8.7×10^{-6}	7.7×10^{-6}

J. Comparative Pharmacokinetic and Neurochemical Studies on TCP and FTCP

J.1 Tissue levels of TCP and FTCP after administration of TCP and FTCP

Figures 78 to 80 show the brain, blood and liver levels of TCP and FTCP. Peak levels of both drugs in all the tissues were observed at 0.5 hr and the levels decreased in a biexponential manner. Peak levels of TCP in brain and liver were not significantly different from each other ($P>0.1$) but they were each significantly higher (nearly 3 times) than those in blood ($P<0.05$). Half-lives of elimination (β) of TCP in the three tissues indicated the following order in rates of elimination: brain>liver>blood. The availabilities of TCP, as measured by the AUC, were significantly different between brain and blood ($P<0.01$), liver and blood ($P<0.001$), but not between brain and liver ($P>0.3$). In quantitative terms the AUCs of TCP in brain and liver were approximately 4 times higher than that in blood.

Peak levels of FTCP in brain and liver were not significantly different from each other ($P>0.1$), but they were significantly higher (nearly 5 times) than those in blood ($P<0.01$). Elimination of FTCP from liver occurred at a slower rate than in brain and blood. The availability of FTCP as measured by the AUC was not different between brain and liver ($P>0.25$) but the AUC in each of these tissues was significantly higher than that in blood ($P<0.001$).

A comparison of the tissue levels of TCP and FTCP revealed that FTCP attained higher peak levels in brain and liver ($P<0.01$), but not in blood ($P>0.2$). The availability of FTCP was also higher in brain and liver than that of TCP ($P<0.001$) but not in blood ($P>0.2$). From the pharmacokinetic parameters summarized in Table 19, it is evident that FTCP was eliminated at a slower rate than TCP from brain and liver, but in blood the rates of elimination of TCP and FTCP were nearly similar.

Levels of TCP and FTCP were also examined in spleen, kidney, heart and lungs at 30 min after administration of equimolar doses, and the results are presented in Fig. 81. Significantly higher tissue levels of FTCP than of TCP were attained in heart, lung and spleen, whereas in kidney levels of both drugs were similar ($P>0.2$).

J.2 Neurochemical effects of TCP and FTCP

In vivo inhibition of brain and liver MAO-A and -B was determined over a 24 hr period following the administration of FTCP and TCP, and the results are presented in Tables 20 and 21. More than 90% inhibition of MAO-A and -B forms was attained within 1.5 hr of administration of each drug, and this remained more or less unchanged over the 24 hr period.

The levels of PEA, T, NA, DA and 5-HT were also measured to determine neurochemical effects of TCP and FTCP. Brain, blood and liver levels of PEA are shown in Figs. 82, 83, and 84. There were significant time-dependent changes in brain PEA levels following the administration of TCP and FTCP [$F(7,34)=6.53$, $P<0.001$ and $F(7,39)=4.12$, $P<0.005$ respectively]. These levels increased 25 fold over control values by 6 hr after injection of TCP and had decreased to 3 times controls by 24 hr. After FTCP administration, peak PEA levels of 40 times controls were obtained at 12 hr and these concentrations decreased to 10 times control values by 24 hr.

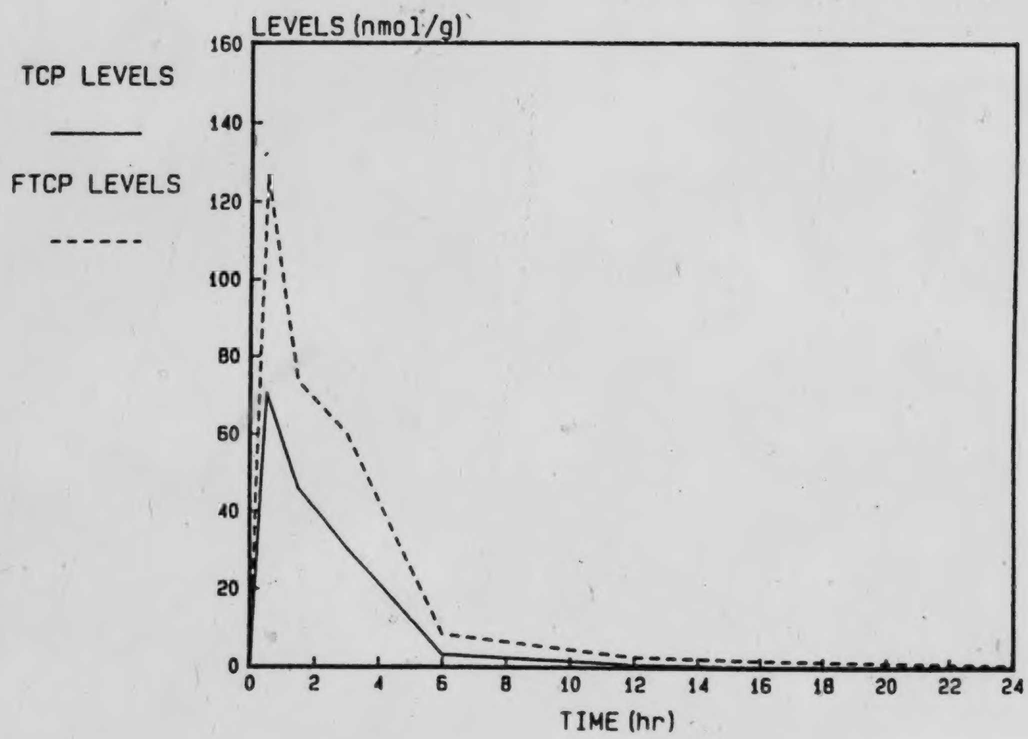


Fig. 78. Rat brain TCP and FTCP levels (nmol/g, mean, n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). S.E.M. values were less than 10% of mean values.

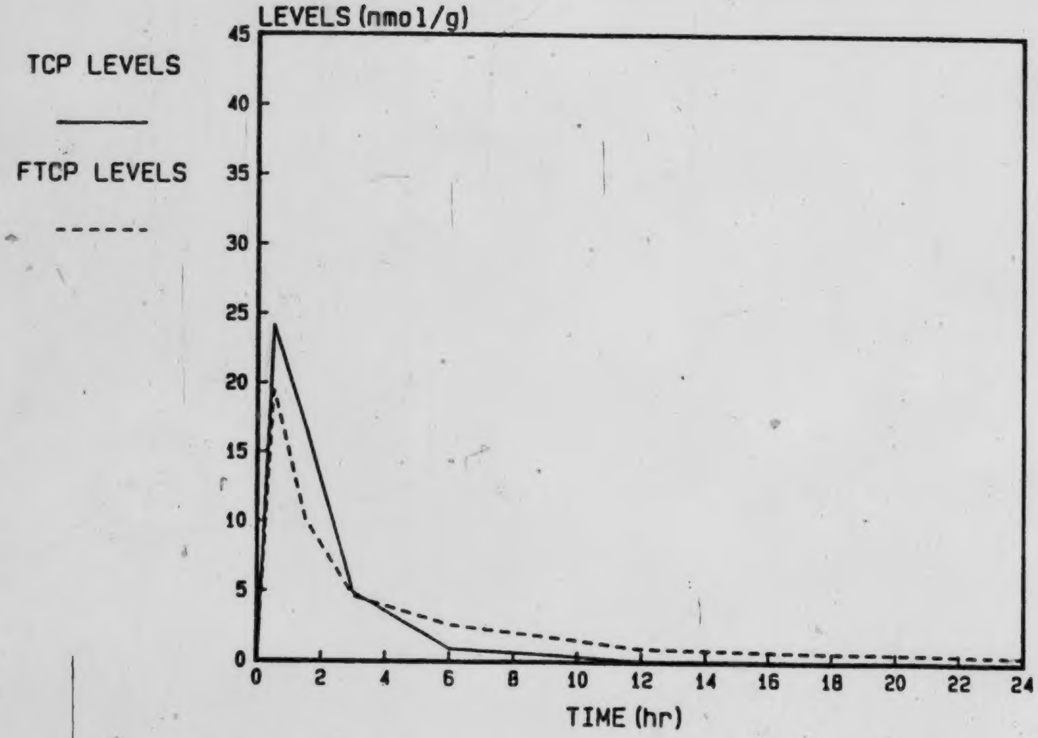


Fig. 79. Rat blood TCP and FTCP levels (nmol/g, mean ± S.E.M., n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). S.E.M. values were less than 10% of mean values.

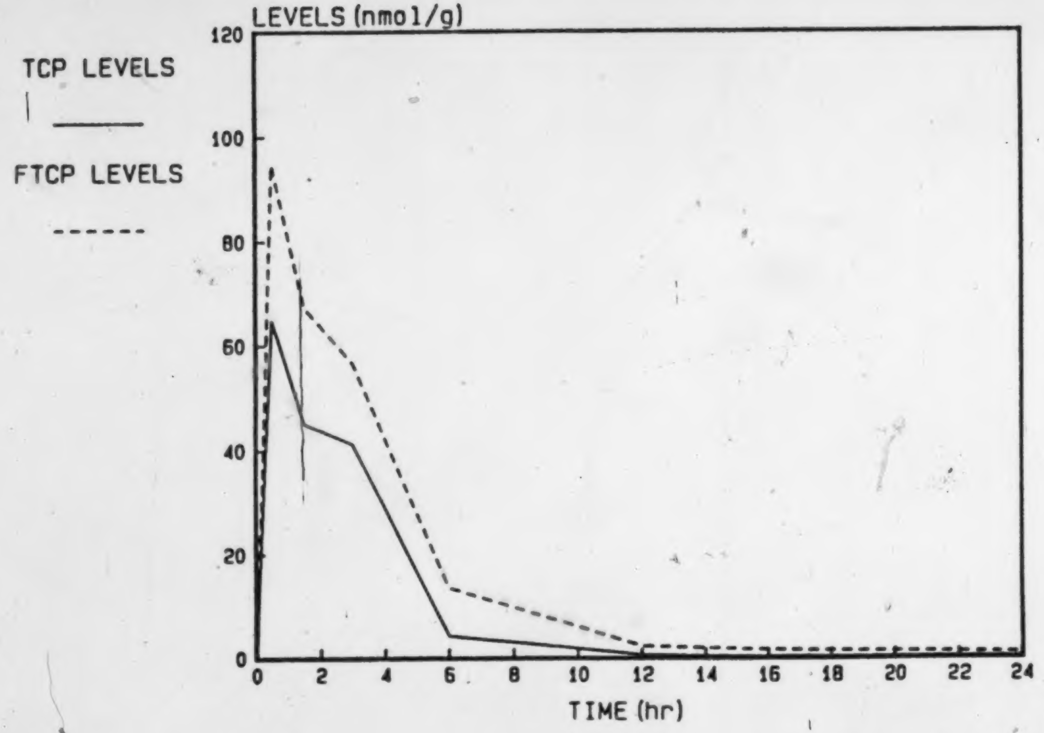


Fig. 80. Rat liver TCP and FTCP levels (nmol/g, mean, n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). S.E.M. values were less than 10% of mean values.

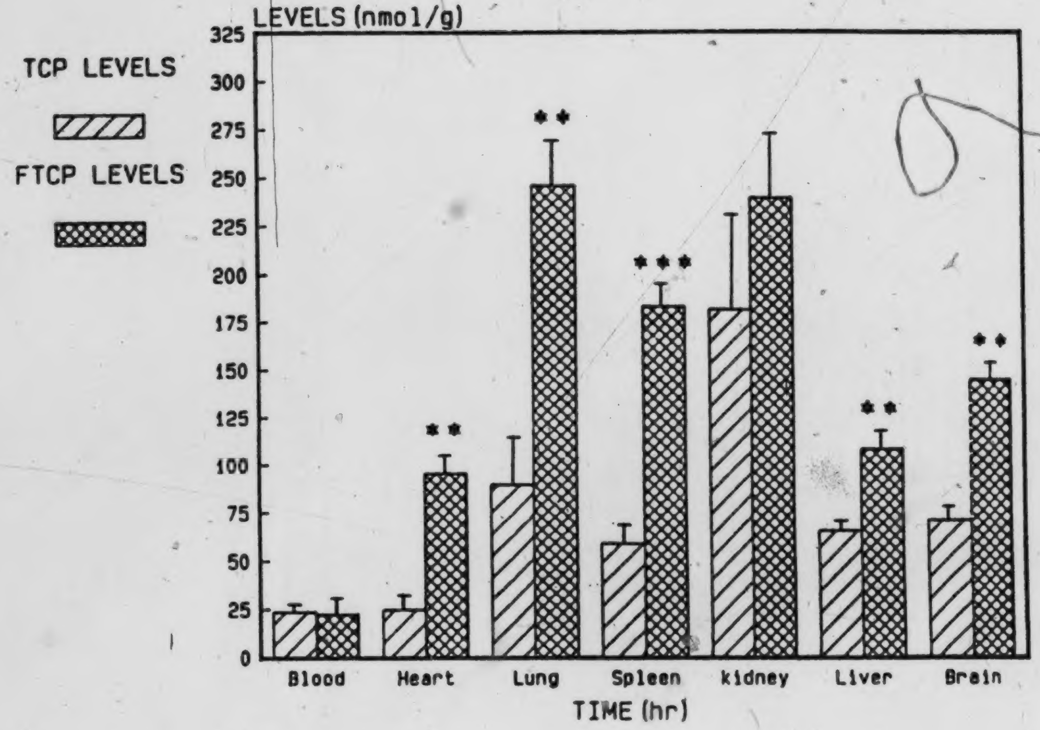


Fig. 81. Tissue TCP and FTCP levels (nmol/g, mean ± S.E.M., n=4-6) after administration of TCP and FTCP (0.1 mmol/kg) (*=$P<0.05$, **=$P<0.01$, ***=$P<0.001$, indicate significant differences between two drug-treated groups).

Table 19. Pharmacokinetic parameters of TCP and FTCP in rat tissues

Drug	Tissue	AUC^a (nmol.g^{-1} hr)	Half-life of Distribution (α) (hr)	Half-life of Elimination (β)(hr)	C_{max}^{a} (nmol/g)
TCP	Blood	57.1 ± 6.5	1.4	11.2	24.1 ± 2.0
	Brain	193.9 ± 15.0	1.0	3.0	70.7 ± 6.8
	Liver	212.0 ± 24.1	1.0	7.7	64.7 ± 4.4
FTCP	Blood	58.3 ± 9.3	0.80	9.4	19.2 ± 6.2
	Brain	376.8 ± 11.9	0.80	7.0	126.5 ± 13.2
	Liver	370.2 ± 20.5	0.60	11.5	94.7 ± 7.9

(a = Mean ± S.E.M., n=5-6)

Table 20. *In vivo* inhibition of rat brain and liver MAO after injection of FTCP (0.1 mmol/kg, i.p.)

% Inhibition (Mean ± S.E.M., n=6 experiments in each case)

	Brain		Liver	
Time (hr)	MAO-A	MAO-B	MAO-A	MAO-B
0.5	80.7 ± 3.8	95.9 ± 0.5	97.3 ± 2.4	98.3 ± 0.1
1.5	90.6 ± 1.8	93.1 ± 0.8	95.4 ± 1.9	98.5 ± 0.1
3.0	86.5 ± 2.2	96.1 ± 1.0	96.1 ± 0.5	98.3 ± 0.2
6.0	96.4 ± 1.1	94.0 ± 1.3	96.8 ± 0.5	97.0 ± 0.1
12.0	90.0 ± 2.5	95.3 ± 1.2	91.4 ± 1.7	96.0 ± 0.5
16.0	86.5 ± 2.0	91.8 ± 3.8	92.8 ± 0.3	92.5 ± 0.9
24.0	86.4 ± 3.9	86.8 ± 4.2	82.8 ± 4.2	89.6 ± 4.6

Table 21. *In vivo* inhibition of rat brain and liver MAO after injection of TCP (0.1 mmol/kg, i.p.).

	% Inhibition (Mean ± S.E.M., n=6 experiments in each case)			
	Brain		Liver	
Time (hr)	MAO-A	MAO-B	MAO-A	MAO-B
0.5	84.9 ± 5.7	97.8 ± 0.3	91.2 ± 4.4	97.2 ± 0.5
1.5	87.4 ± 3.2	95.4 ± 0.7	92.5 ± 3.0	97.8 ± 0.3
3.0	92.0 ± 2.0	96.8 ± 0.4	93.8 ± 1.4	93.4 ± 2.6
6.0	91.3 ± 1.3	95.8 ± 0.7	96.5 ± 0.3	96.5 ± 0.8
12.0	93.2 ± 0.6	94.0 ± 1.0	95.1 ± 0.5	96.5 ± 0.3
16.0	85.2 ± 3.0	91.6 ± 0.3	83.6 ± 1.3	91.4 ± 0.5
24.0	90.2 ± 3.7	93.4 ± 0.8	83.7 ± 0.7	91.6 ± 0.6

Significant time-dependent changes in blood PEA levels were observed following the administration of TCP and FTCP [F(7,26)=88.24, P<0.001 and F(7,38)=6.59, P<0.001 respectively]. Following TCP administration, PEA levels increased to nearly 30 times control levels within 0.5 hr and decreased to about 4 times control levels by 1.5 hr and to approximately control levels by 12 hr (Fig. 83). After FTCP administration, blood PEA levels increased gradually, and peak levels nearly 20 times control levels were attained by 3 hr; even after 24 hr the levels were 6 times control levels (Fig. 83).

Significant time-dependent changes in liver PEA levels were observed after the administration of TCP [F(7,34)=6.06, P<0.001] and FTCP [F(7,35)=3.82; P<0.05]. Details are given in Fig. 84. Peak PEA levels that were nearly 20 times control levels were attained at 6 hr after TCP. These decreased to nearly 6 times control values by 24 hr. Peak PEA levels after FTCP treatment were nearly 40 times control values at 12 hr and decreased to approximately 13 times control values by 24 hr.

Levels of PEA in rat tissues 30 min after administration of TCP and FTCP along with control levels are presented in Table 22. Values for PEA in control tissues measured by TLC-HRMS (Philips, 1984) are included in this table for comparative purposes. There were marked increases in PEA concentrations in all tissues after treatment with TCP and FTCP.

Levels of T in brain and liver are shown in Figs. 85 and 86. The percentage increases in T levels in comparison to control values were higher than with PEA levels. With the present method of analysis of T, control levels could not be accurately quantitated in any organ but they were <1 ng/g. The reported levels of T in rat brain and liver are 0.5

and 0.7 ng/g respectively using a high resolution GC-MS procedure (Philips, 1974, 1984). Peak levels that were nearly 200 times these control levels were attained in brain 1.5 hr after TCP administration. These decreased to about 20 times control values by 12 hr, after which there was a marked decrease in T levels. At 16 and 24 hr T could be detected in only one brain in a group of 4-6. After FTCP administration brain, T levels attained a peak at 3 hr (comparable to peak levels after TCP), and by 24 hr the levels were nearly 18 times control values.

The increase in T levels in liver was more pronounced than in brain. Significant time-dependent changes in liver T levels following the administration of TCP and FTCP were demonstrated [$F(6,27)=4.69$, $P<0.005$ and $F(6,33)=6.37$, $P<0.001$ respectively]. After TCP, peak T levels were nearly 285 times control values at 3 hr and decreased to nearly 18 times control values by 24 hr. After FTCP, peak levels representing a 180-fold increase over control levels were attained at 1.5 hr; by 24 hr these concentrations had decreased to nearly 50 times control values. Because of the problems with sensitivity and interfering peaks, a comprehensive investigation of T in blood samples was not possible.

Brain NA, DA and 5-HT levels are shown in Figs. 87, 88 and 89. There were significant time-dependent changes in NA levels after the administration of TCP and FTCP [$F(7,32)=42.44$, $P<0.001$ and $F(7,46)=27.87$, $P<0.001$ respectively]. Following TCP administration, NA levels decreased below control levels at 0.5 and 1.5 hr ($P<0.05$) but increased significantly above the control levels steadily after 3 hr ($P<0.05$); by 24 hr the levels were about 1.6 times control levels. After FTCP injection, NA levels increased significantly above control levels only after 6 hr and by 24 hr they were nearly 1.5 times control levels.

Significant time-dependent changes in brain DA levels after the administration of TCP and FTCP were noted [F(7,32)=25.44, $P<0.001$ and F(7,46)= 8.09, $P<0.001$ respectively]. Following TCP administration, DA levels increased to nearly 2.4 times control levels by 3 hr; by 24 hr control levels were observed. Levels at 3, 6 and 12 hr were significantly above control values. After FTCP, peak DA levels at 0.5 hr were about 1.5 times control levels and remained well above the control levels even after 24 hr ($P<0.05$).

There were significant time-dependent changes in 5-HT levels after the administration of both drugs [F(7,32)=24.83, $P<0.001$ and F(7,46)= 36.09, $P<0.001$ respectively]. Levels of 5-HT increased to nearly 3 times control levels by 6 hr and had decreased to 1.7 times control values by 24 hr after TCP treatment. Levels of 5-HT over the entire 24 hr period were significantly above the control values ($P<0.05$). Injection of FTCP caused 5-HT levels to increase much more slowly; peak levels at 16 hr were nearly 2.5 times control values and by 24 hr the levels were approximately 1.6 times control values. Levels from 6 to 24 hr were significantly higher than control values.

K. Pharmacokinetic and Neurochemical Studies on PGTCP

The N-propargyl analog of TCP was examined as a possible prodrug of the MAO-inhibiting antidepressant TCP. *In vitro* data on inhibition of MAO (Table 18) indicated that PGTCP was approximately 100 times and 85 times weaker an inhibitor of MAO-A and -B, respectively, than was TCP. Levels of PGTCP and TCP were measured in rat brain, blood and liver and the effects of PGTCP administration on levels of PEA, NA, DA and 5-HT

Table 22. Levels of PEA[a] in the tissue of control rats and in rats treated 30 min previously with TCP and FTCP (0.1 mmol/kg i.p. each).

Tissue	Controls	TCP-Treated	FTCP-Treated	Control Levels Using TLC-HRMS[b]
Blood	2.5 ± 0.31	77.7 ± 7.8	11.1 ± 2.7	1.9
Brain	1.9 ± 0.36	28.5 ± 2.9	54.0 ± 10.9	1.8
Liver	3.2 ± 0.48	54.6 ± 8.4	88.2 ± 25.0	2.0
Lung	5.1 ± 1.2	21.3 ± 3.1	14.9 ± 2.1	4.0
Kidney	22.0 ± 1.6	141.2 ± 31.3*	46.1 ± 2.8	20.5
Heart	3.4 ± 1.7	17.8 ± 2.5	20.6 ± 3.0	5.7
Spleen	3.8 ± 1.1	18.8 ± 1.3	17.5 ± 3.3	4.7

[a]=ng/g, mean ± S.E.M., n=4-8

[b]=Data from Philips (1984)

*=different from FTCP-treated group, $P<0.05$ (significant differences in brain, blood and liver PEA levels between TCP- and FTCP-treated groups are presented in Figs. 82-84).

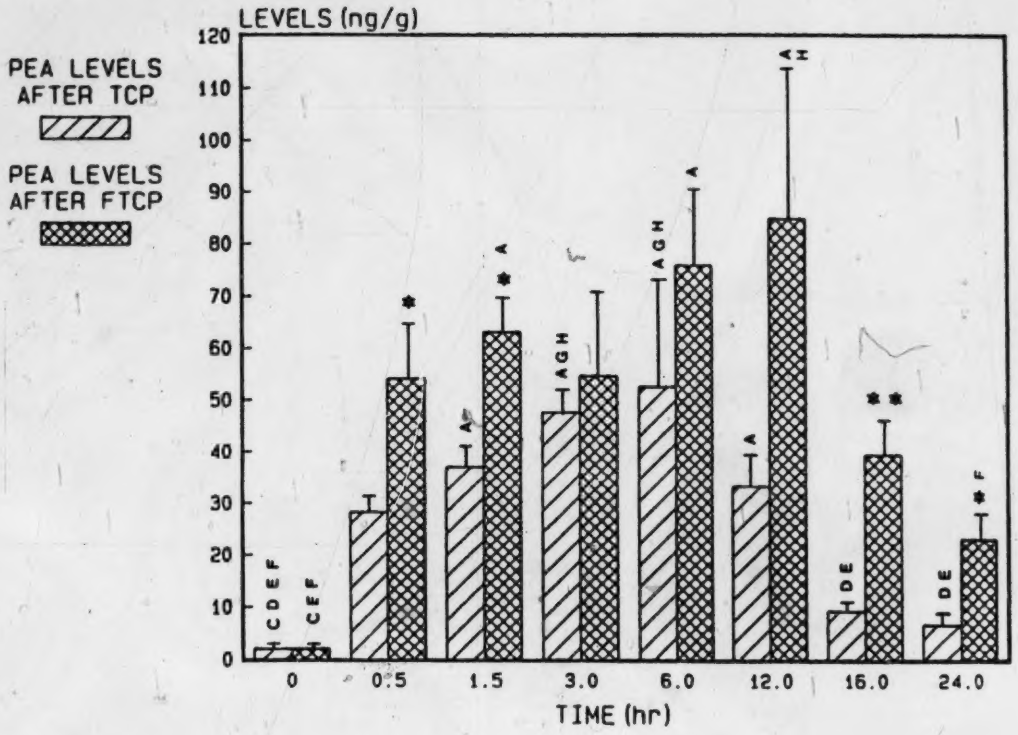


Fig. 82. Rat brain PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of TCP and PFTCP (0.1 mmol/kg). Superscripts A, B, C, D, E, F, G and H indicate significant differences from levels at 0, 0.5, 1.5, 3.0, 6.0, 12.0, 16.0 and 24.0 hr respectively (α=0.05). (*=P<0.05, **=P<0.001, ***=P<0.001 indicate significant differences between two drug-treated groups).

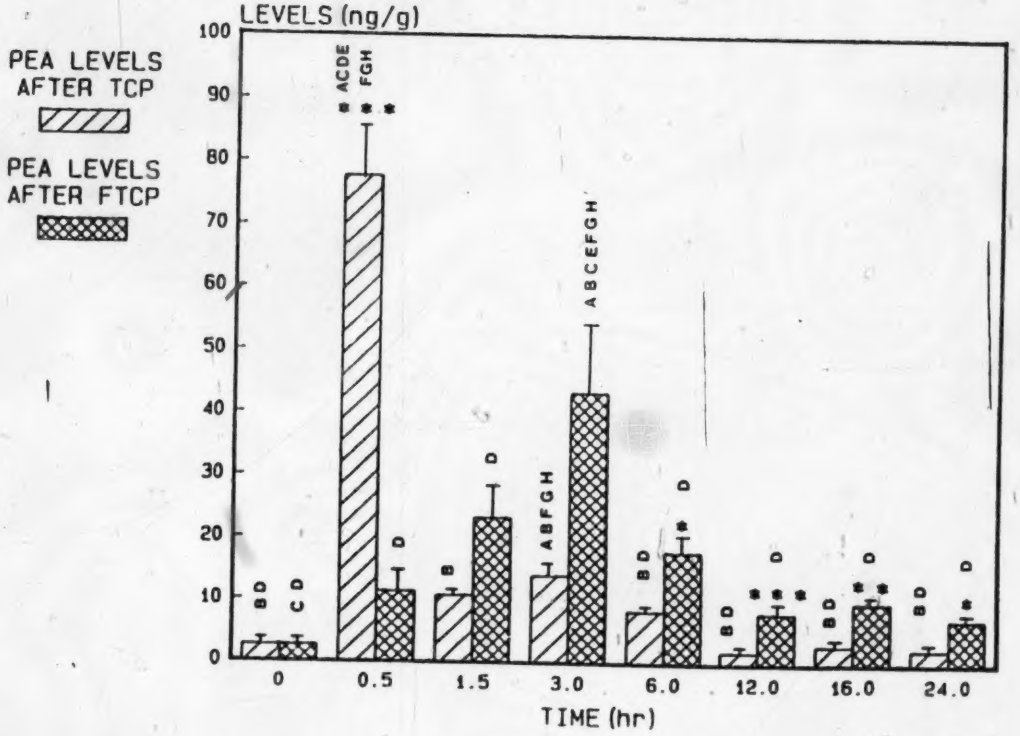


Fig. 83. Rat blood PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

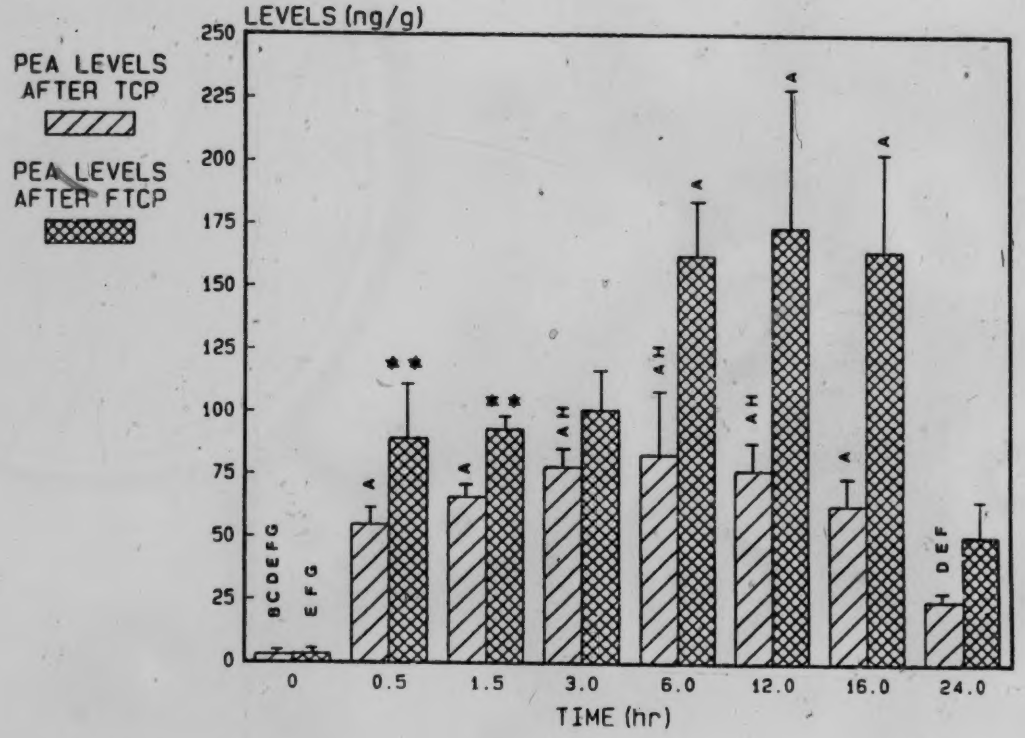


Fig. 84. Rat liver PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

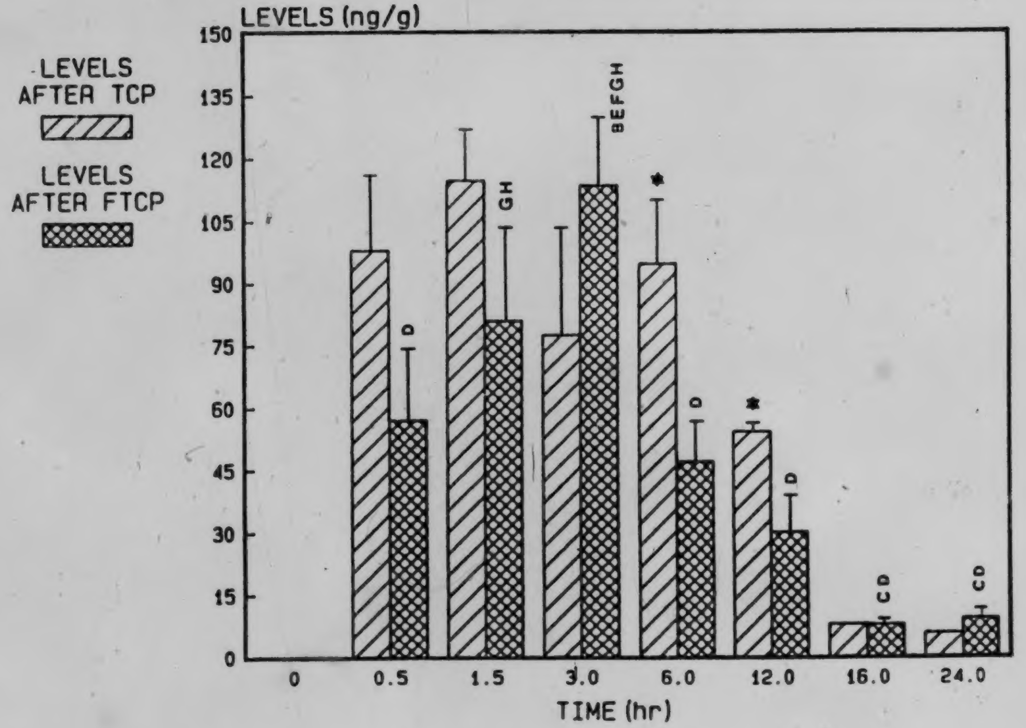


Fig. 85. Rat brain T levels (ng/g, mean ± S.E.M., n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

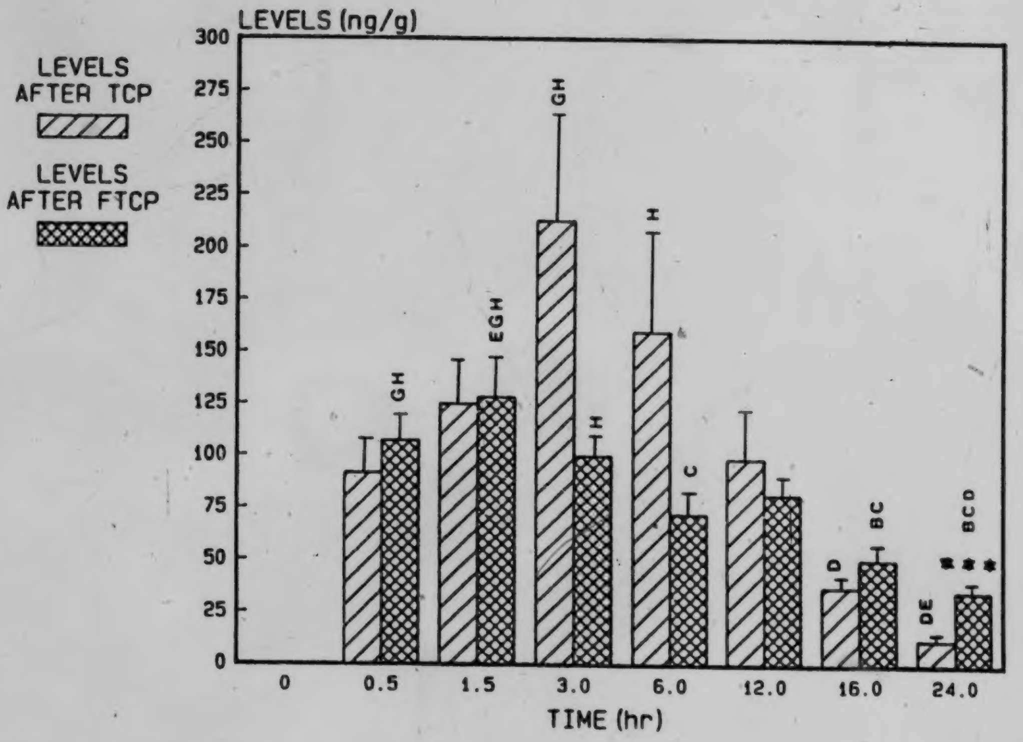


Fig. 86. Rat liver T levels (ng/g, mean ± S.E.M., n=5-6) after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

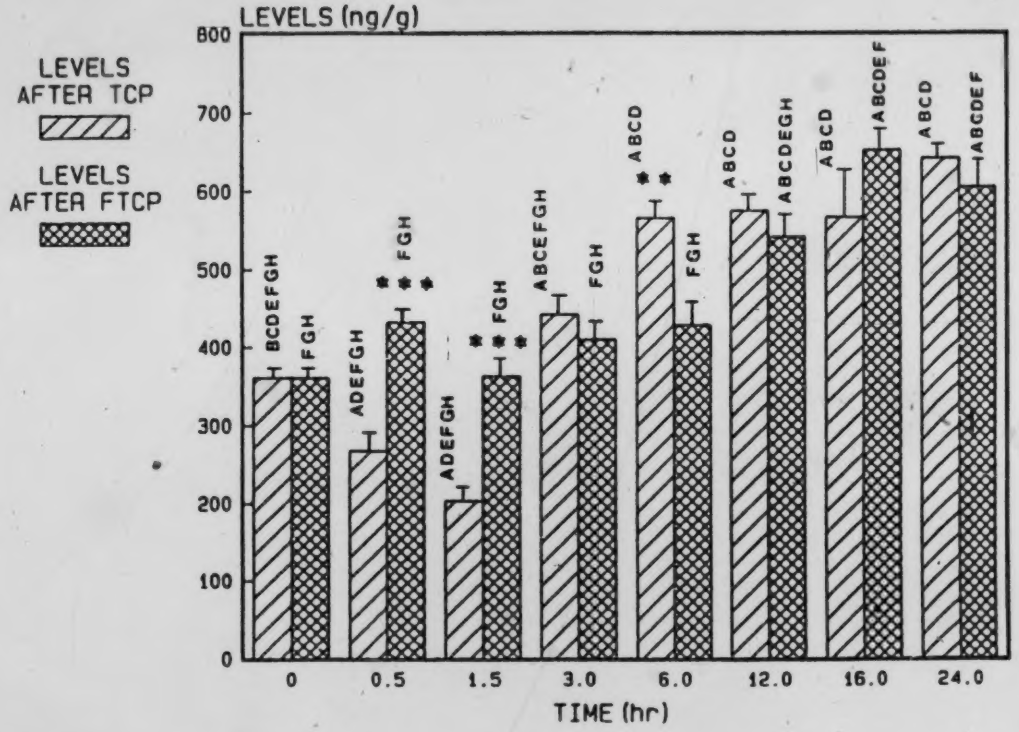


Fig. 87. Rat brain NA [ng/g, mean ± S.E.M., n=5-6 (drug-treated), n=12 (controls)] levels after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

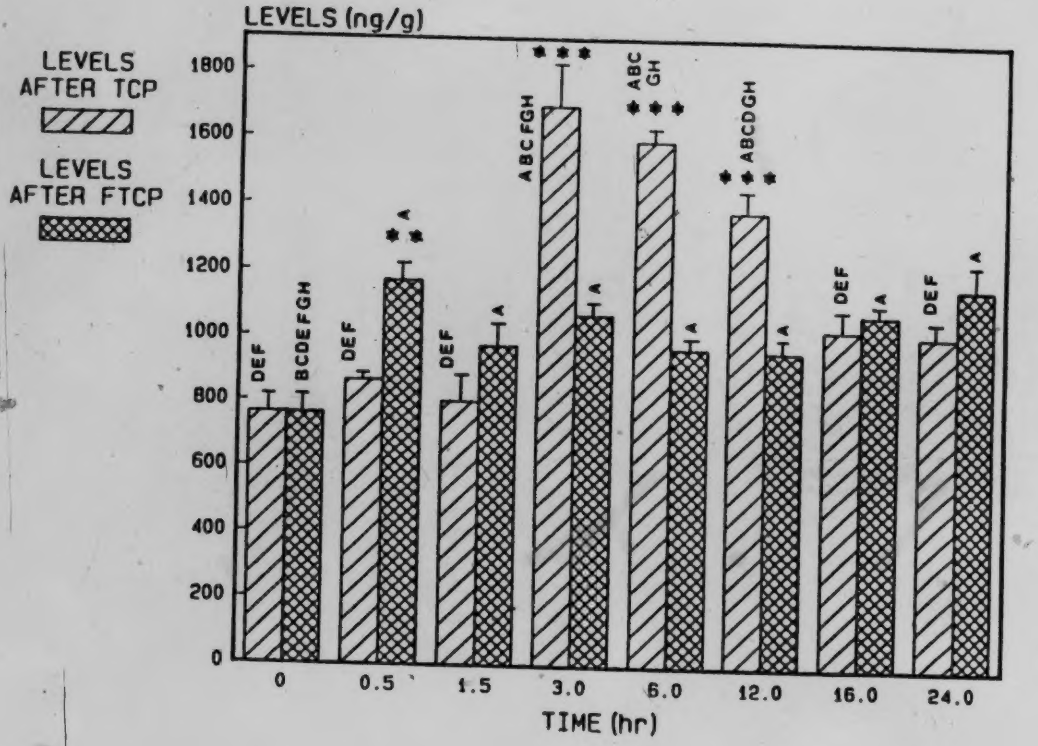


Fig. 88. Rat brain DA levels [ng/g, mean ± S.E.M., n=5-6 (drug-treated), n=12 (controls)] after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts befer to Fig. 82.

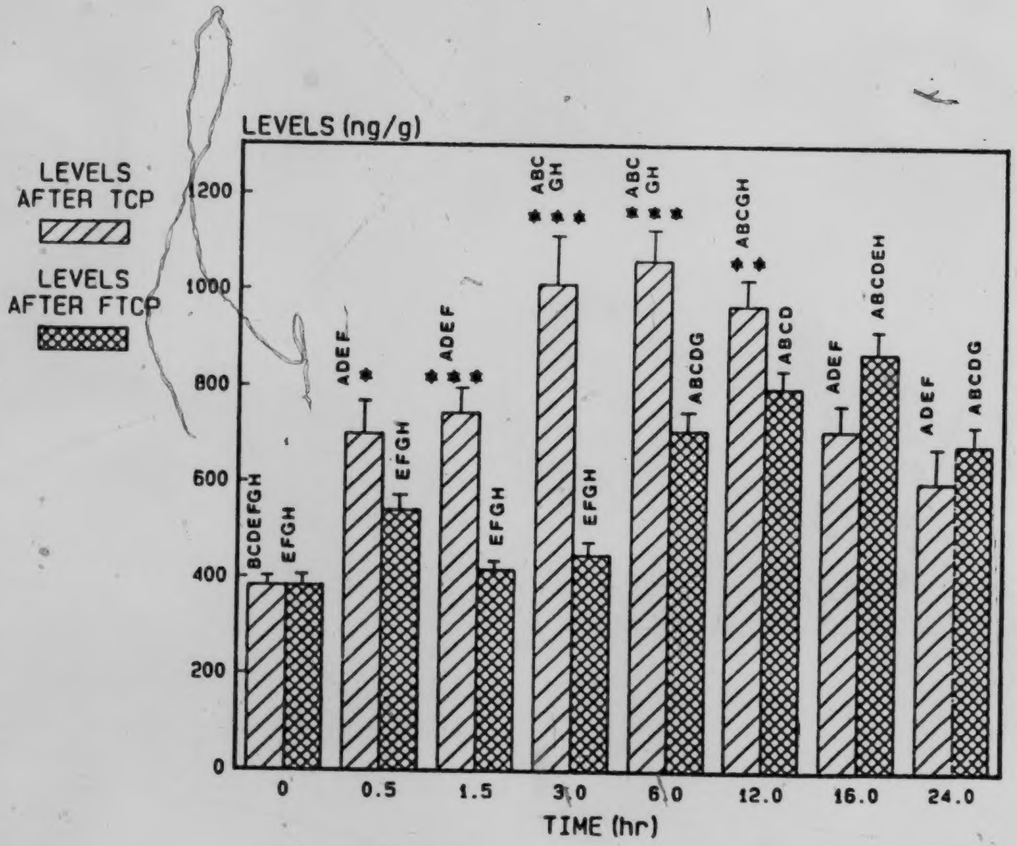


Fig. 89. Rat brain 5-HT levels [ng/g, mean ± S.E.M., n=5-6 (drug-treated), n=12 (controls)] after administration of TCP and FTCP (0.1 mmol/kg). For statistical notations and superscripts refer to Fig. 82.

were examined. In vivo inhibition of MAO-A and -B in brain and liver over a 24-hr period was also studied.

K.1 Levels of PGTCP and TCP in rat brain, blood and liver following the administration of PGTCP

Peak levels of PGTCP were observed at 0.5 hr in all three tissues (Fig. 90). Peak levels in liver were nearly 25 times higher ($P<0.001$) and 8.5 times higher ($P<0.001$) than those in brain and blood respectively. Levels in all three tissues decreased monoexponentially and the elimination half-lives in all the three tissues were similar (Table 23). Levels of PGTCP were not detectable beyond 16 hr after administration. The availability of PGTCP as measured by the AUC was significantly different between brain and liver ($P<0.001$), brain and blood ($P<0.005$) and blood and liver ($P<0.001$). In quantitative terms, the availability in liver was nearly 18 times and 10 times higher than that in brain and blood respectively.

The dealkylated metabolite of PGTCP, namely TCP, was also measured in these tissues and the levels are presented in Fig. 91. Peak levels of TCP in these tissues were observed at 0.5 hr. The levels in liver were nearly 4 times and 10 times higher than those in brain and blood respectively ($P<0.05$). Levels of TCP decreased with similar half-lives in brain, blood and liver and in all three tissues the levels of TCP were undetectable beyond 12 hr. The AUC values indicated that that availability of TCP in liver after administration of PGTCP was nearly 2.5 times ($P<0.001$) and 5 times ($P<0.001$) that in brain and blood respectively. The AUC of TCP in brain was also significantly higher than that in blood ($P<0.001$). The pharmacokinetic parameters are summarized in Table 23.

The *in vivo* inhibition of MAO-A and -B in brain and liver after PGTCP treatment is presented in Table 24. In brain and liver nearly 85% inhibition of MAO-A and -B was attained by 3 hr (although MAO-B inactivation appeared to be faster) and by 24 hr nearly 75% of both MAO-A and -B remained inhibited in brain. In liver, inhibition of MAO-A was nearly 60% at 24 hr, with MAO-B inhibition around 78% of the control level at the same time interval.

K.2 Levels of PEA, NA, DA and 5-HT after PGTCP administration

There were significant time-dependent changes in PEA levels in brain and liver [$F(7,38)=39.87$, $P<0.001$ and $F(7,39)=31.96$, $P<0.001$ respectively]. Levels of PEA in brain, blood and liver are presented in Fig. 92. Peak levels of PEA in brain at 1.5 hr were nearly 8 times the control values and had decreased to control values by 16 hr. Peak levels of nearly 14 times control values were observed in liver at 3 hr, and these decreased to control values by 16 hr. Blood concentrations of PEA after administration of PGTCP were not significantly different from control values.

The findings with NA, DA and 5-HT are presented in Fig. 93. There were significant time-dependent changes in levels of all three amines [$F(7,45)=78.10$, $P<0.001$; $F(7,44)=3.44$, $P<0.025$ and $F(7,45)=34.48$, $P<0.001$) respectively]. Levels of NA were increased significantly above control levels at all time intervals from 1.5 to 24 hr while levels of DA decreased significantly below control levels at 0.5 hr and thereafter increased to control levels. Significant elevations of 5-HT were observed from 0.5-24 hr and peak levels were attained at 6 hr. Levels of the trace amine T could not be accurately measured because of sensitivity problems.

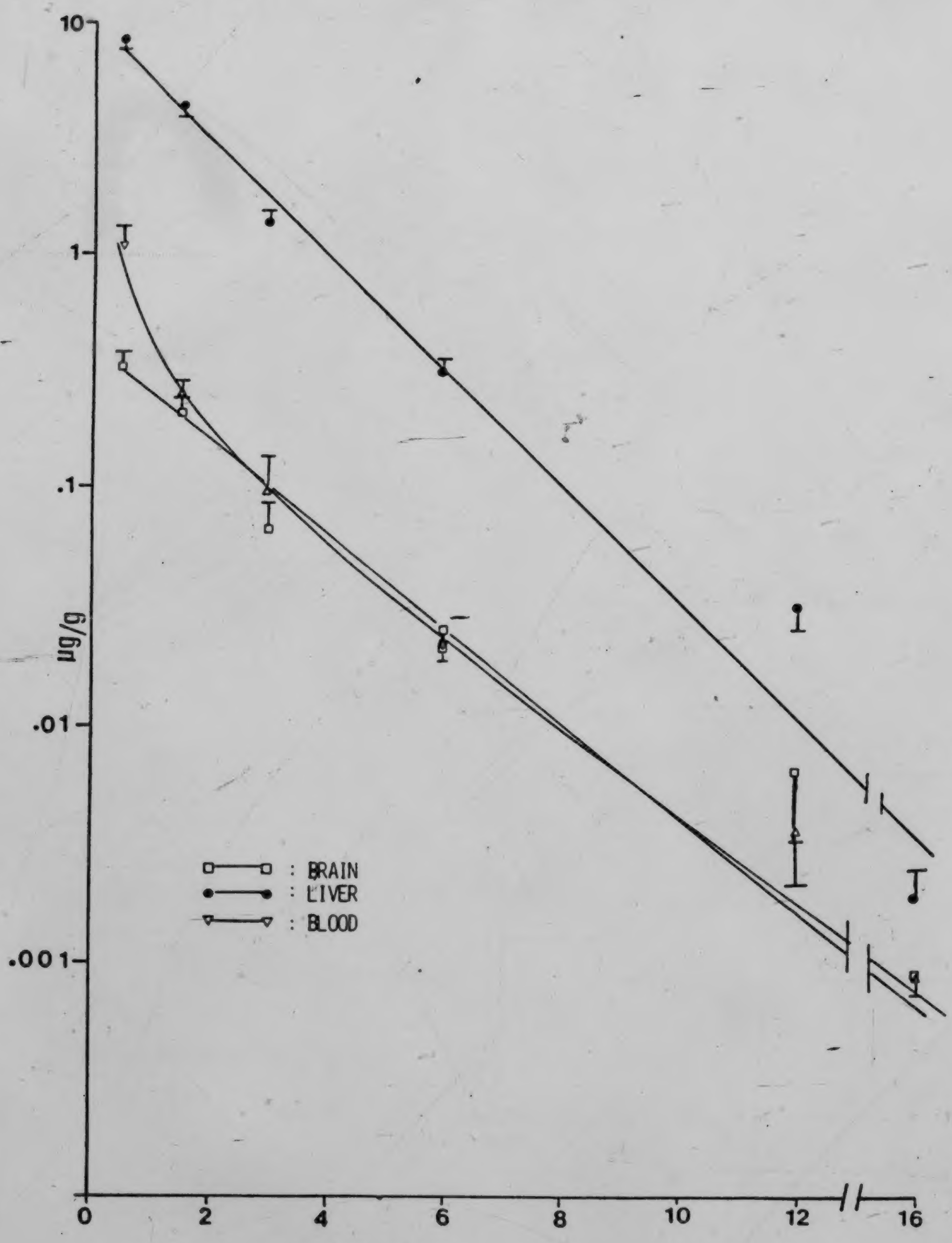


Fig. 90. Rat brain (□), blood (△) and liver (●) PGTCP levels (µg/g, mean ± S.E.M., n=5-6) after administration of PGTCP (0.1 mmol/kg, i.p.).

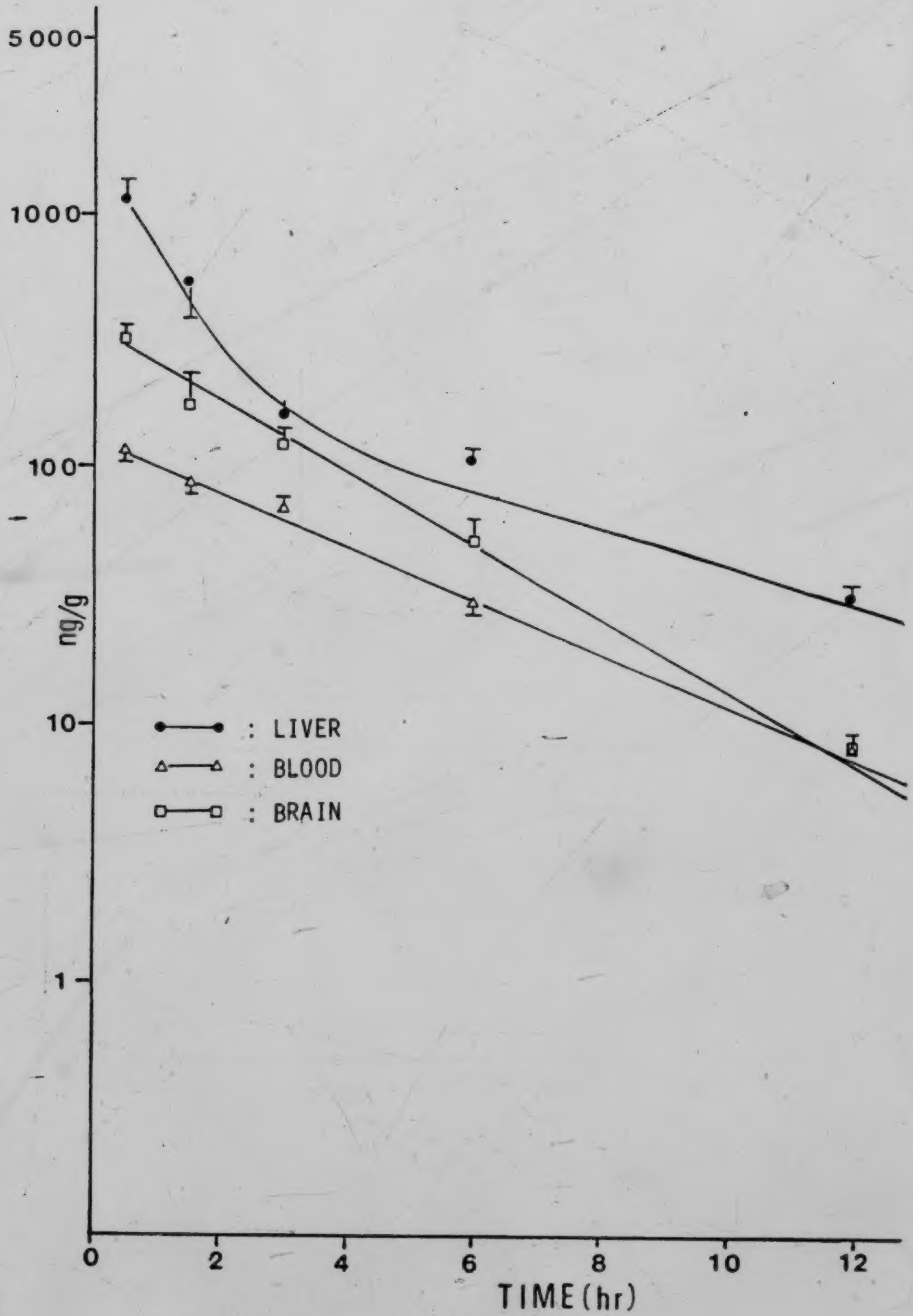


Fig. 91. Rat brain (□), blood (△) and liver (●) TCP levels (ng/g, mean ± S.E.M., n=5-6) after administration of PGTCP.

Table 23. Pharmacokinetic parameters of TCP and PGTCP in rat tissues.

Drug	Tissue	AUC[a] (nmol.g^{-1} hr)	Half-life of Elimination ($t_{1/2}$)(hr)	C_{max}[a] (nmol/g)
TCP from PGTCP	Brain	7.6 ± 0.28**	2.0	2.49 ± 0.41
	Blood	3.9 ± 0.14**	3.0	0.87 ± 0.04
	Liver	19.5 ± 1.9**	2.9	9.0 ± 0.76
PGTCP	Brain	4.8 ± 0.42*	1.5	1.9 ± 0.29
	Blood	8.2 ± 0.92*	1.6	6.13 ± 0.99
	Liver	88.5 ± 7.7*	1.2	49.7 ± 4.1

[a]=mean ± S.E.M. (n=5-6)
*=0-16 hr
**=0-12 hr

Table 24. Inhibition of rat brain and liver MAO in vivo after injection of PGTCP (0.1 mmol/kg, i.p.).

	% Inhibition (Mean ± S.E.M, N=6 experiments in each case)			
	Brain		Liver	
Time (hr)	MAO-A	MAO-B	MAO-A	MAO-B
0.5	73.1 ± 3.0	93.6 ± 0.61	75.14 ± 1.4	90.8 ± 0.50
1.5	74.8 ± 2.2	90.1 ± 1.1	83.3 ± 2.0	92.2 ± 0.23
3.0	84.8 ± 2.0	96.0 ± 0.43	87.7 ± 0.20	92.3 ± 0.24
6.0	89.1 ± 1.2	95.5 ± 1.4	89.4 ± 0.94	93.4 ± 0.40
12.0	85.1 ± 1.0	93.6 ± 0.5	80.6 ± 0.80	88.0 ± 0.32
16.0	82.6 ± 1.1	91.9 ± 1.0	76.1 ± 1.3	85.1 ± 0.44
24.0	75.2 ± 1.5	84.0 ± 1.4	55.6 ± 1.5	78.0 ± 0.83

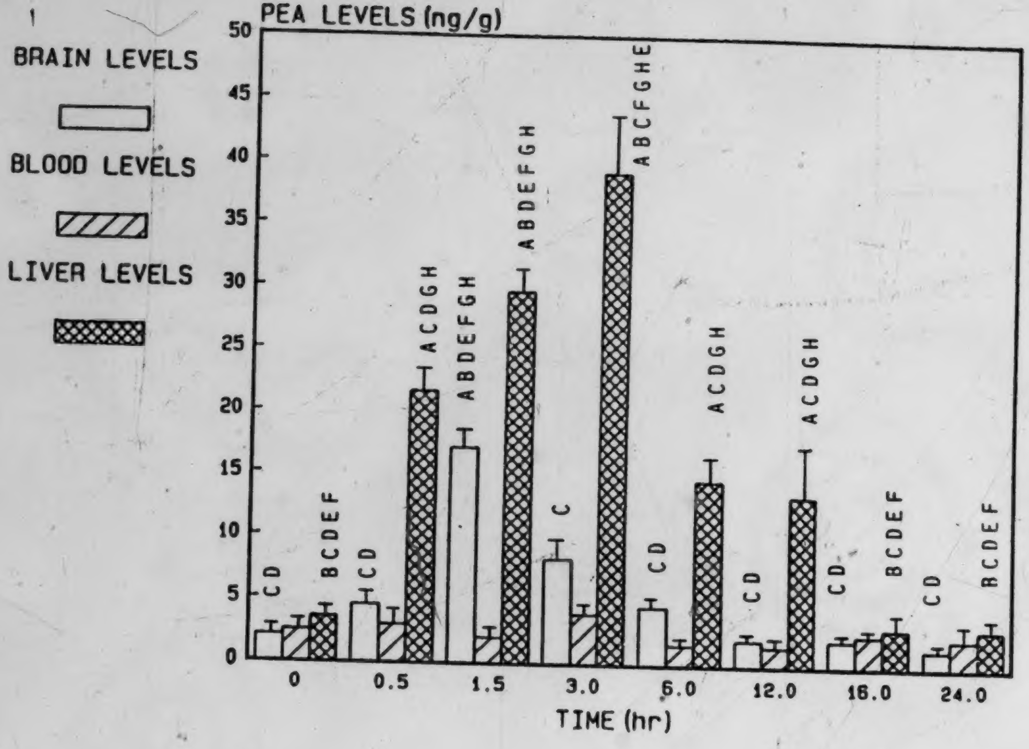


Fig. 92. Rat brain, blood and liver PEA levels (ng/g, mean ± S.E.M., n=5-6) after administration of PGTCP (0.1 mmol/kg). Superscripts A, B, C, D, E, F, G and H indicate significant differences from levels at 0, 0.5, 1.5, 3.0, 6.0, 12.0, 16.0 and 24.0 hr respectively (α=0.05).

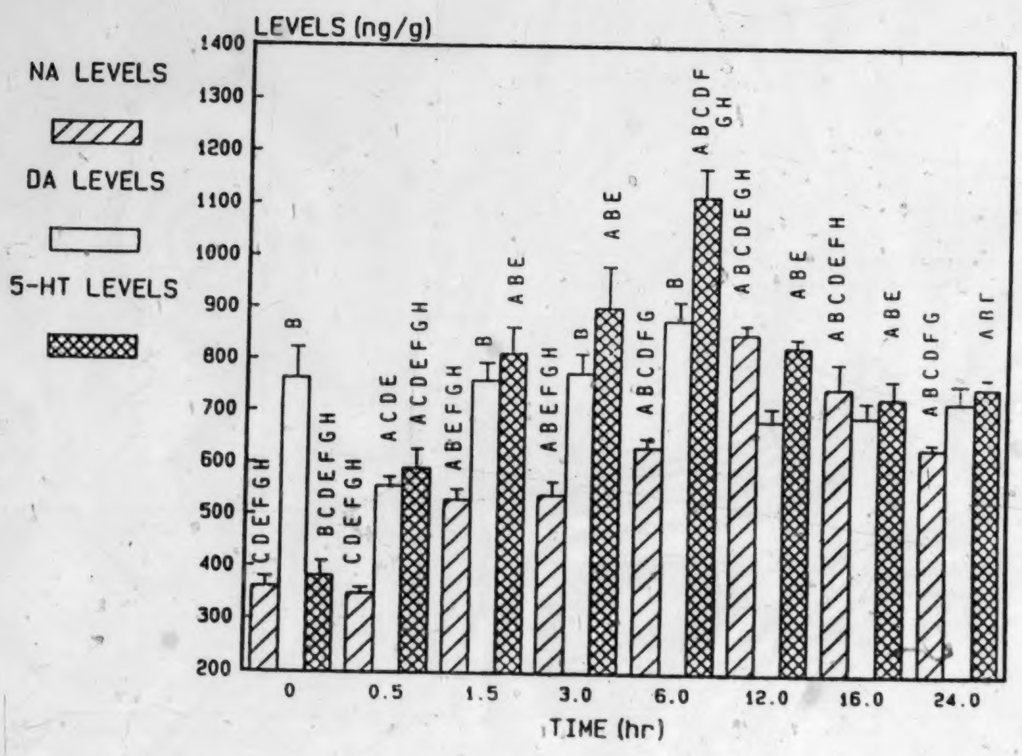


Fig. 93. Rat brain NA, DA and 5-HT levels (ng/g, mean ± S.E.M., n=5-6 (drug-treated), n=12 (controls)] after administration of PGTCP (0.1 mmol/kg). For superscripts refer to Fig. 92.

L. Analysis of PLZ in Rat Brain and in Patient Urines by Extractive Pentafluorobenzoylation

Rat brain levels of the MAO inhibitor PLZ 1, 2, 4 and 8 hr after administration of 15 mg/kg PLZ were [ng/g, mean ± S.E.M., (n)] 45.4 ± 9.7 (5), 35.6 ± 8.3 (5), 27.1 ± 3.5 (9), and 15.8 ± 2.7 (5) respectively. Brain levels 4 hr after 30 or 60 mg/kg PLZ administration (dose based on free base) were [ng/g, mean ± S.E.M., (n)] 73.7 ± 17.2 (5) and 150.1 ± 40.2 (5) respectively. In 13 patients receiving 15 mg of PLZ sulfate t.i.d. for 2 weeks, the mean excretion of PLZ in 24 hr urine samples was 1211 ± 356 (mean ± S.E.M.) g/24 hr urine.

IV. DISCUSSION

A. Rat brain, blood and liver levels of PEA and its prodrugs

As mentioned in the Introduction (see Section I.L), one of the primary objectives of the project was to investigate the use of analogs of PEA and T as possible prodrugs of these amines. It was hypothesized that these N-alkylated analogs would result in elevated, sustained levels of PEA or T in brain without elevating other biogenic amines such as the catecholamines or 5-HT. In order to provide a comprehensive comparison with the parent amines (PEA and T), these parent amines were injected intraperitoneally in other groups of rats and the levels of PEA or T in brain, liver and blood as well as brain levels of the catecholamines and 5-HT and their metabolites were compared to those found in the rats which had been treated with the potential prodrugs.

A.1 Tissue levels of PEA after administration of PEA

Administration of PEA resulted in marked increases in tissue levels of PEA, with peak levels in all three tissues occurring at 5 min (Figs. 33-35. The profiles in blood and brain were similar, whereas in liver peak levels were lower than those in brain and blood and the rate of decline of PEA levels was also much slower. Peak levels of PEA in brain at 5 min indicate its rapid entry into the brain and its ability to cross the blood-brain barrier with relative ease. Nakajima *et al.* (1964) observed that PEA levels in MAO inhibitor-treated rabbit brains exceeded the plasma levels at 5 min following PEA injection (10 mg/kg, i.v.). Oldendorf (1971) determined the brain uptake index (BUI) of PEA to be 67 compared to 100 for $[^3H]-H_2O$, indicating rapid penetration into brain. Significant levels of $[^{14}C]$-PEA in brain at 15 sec following PEA administration (intracarotid infusion) were observed by Greenberg and Whalley (1978). Wu and Boulton (1975) reported peak levels of PEA at 5 min following i.v. injection of PEA. In contrast to the above reports indicating rapid entry into brain, Edwards and Antelman (1978) reported peak PEA levels in brain 20 min after PEA injection (100 mg/kg, i.p.) and Cohen _et_ _al._ (1974) found peak levels of PEA at 15 min in brain, and at 5 min in blood and liver following PEA administration (40 mg/kg, i.p.). These two reports are at variance with the known lipophilicity and rapid penetration of PEA across the blood-brain barrier.

Following a marked transient increase, PEA levels in brain and blood decreased very rapidly and at a slower rate in liver. It has been reported that PEA is an excellent substrate for MAO-B enzyme (Yang and Neff, 1973); Wu and Boulton (1975) reported detection of phenylacetic acid (PAA) as a major metabolite of PEA in various tissues and urine following PEA administration without any MAO inhibitor pretreatment. The rapid decline in brain PEA levels could be due to rapid metabolism and/or redistribution into various tissues. Although PEA elimination in brain exhibited a slower elimination phase (β) following a rapid elimination phase (α), the β-phase would be of little pharmacokinetic importance since the rapid decline phase accounted for more than 90% of the total PEA available to brain. Cohen _et_ _al._ (1974) reported a half-life of less than 15 min in brain, blood and liver with no traces of PEA in these tissues at 30 min following a 40 mg/kg (i.p.) dose. In a study on PEA levels in rat tissues 30 min after an i.v. injection, Wu and Boulton (1975) reported recovery of PEA only after MAO inhibitor (PAR) pretreatment. Edwards and Antelman (1978) reported detection of small amounts of PEA in caudate nucleus and the rest of the brain 60 min after injection of 100 mg/kg (i.p.). A first-order decline of PEA levels in brain in the presence of PAR was found by Wu and Boulton (1975); there was a seemingly multiexponential decline in the absence of PAR, and the half-lives of PEA were approximately 40 min in whole brain (assuming a linear decline), 1-5 min in different brain regions and 27 min in liver. Shannon et al. (1982) reported a half-life of 5 min for PEA in dog plasma at a dose of 5.6-10.0 mg/kg (i.v.). In the above cited reports PEA levels were not monitored for longer times to characterize the slower elimination phase as was done in the present investigation. The β value of PEA obtained in liver agrees with that reported by Wu and Boulton (1975). Recently, Garcha et al. (1985) observed route of administration-dependent elimination kinetics of PEA in cat. Shannon et al. (1982) reported an increase in half-life from nearly 6 min at a dose of 5.6 mg/kg (i.v.) to 16.0 min at a dose of 17.5 mg/kg (i.v.) ($P < 0.001$).

A preliminary investigation in our laboratories of rat brain and liver levels of PEA at 5 and 15 min after i.p. administration of 0.05 mmol/kg of PEA showed peak levels of PEA in brain and liver which were significantly lower than those expected from the 0.1 mmol/kg dose data. These data suggest that PEA exhibits nonlinear kinetics as well as route of administration-dependent kinetics. The differences in kinetics seen in the present investigation and those reported earlier could possibly be due to differences in doses employed and in the route of administration.

Interestingly, peak levels of PEA in liver were lower than those seen in brain and blood (Figs. 33-35). Liver contains quantitatively higher MAO activity than brain (Gorkin, 1983) and PEA would be expected to be rapidly metabolized in liver. Kinemuchi et al. (1982) reported time-dependent MAO inhibition at high concentrations of PEA and this inhibition was shown not to be due to any of the deaminated metabolites of PEA. Very high levels of PEA available to liver after i.p. administration might saturate and/or inhibit MAO, leading to rapid passive diffusion of available PEA to other tissues. The slower elimination of PEA from liver despite the presence of higher MAO activity and seemingly lower initial PEA levels (in contrast to brain and blood) suggests some form of enzyme saturation and/or inhibition. Despite the differences in peak concentrations and rates of elimination of PEA from brain, blood and liver in the present study, the total overall availability over the time course studied, as measured by AUC, was not different between any two tissues (Table 8).

A.2 Tissue levels of PEA and CEPEA after administration of CEPEA

In contrast to the transient increases in PEA levels after administration of PEA, CEPEA administration caused more gradual and sustained increases in PEA levels. The PEA formed from CEPEA was eliminated more slowly than PEA after PEA administration in brain and blood but not in liver (Figs. 33-35 and Table 8). Analysis of PEA levels in brain after administration of an equimolar dose of PEA and CEPEA indicated a significant difference between effects of drug treatments (i.e., PEA and CEPEA) [F(1,75)=18.64, P<0.001] and of time [F(7,75)=56.30, P<0.001] and a significant interaction between drug treatment and time (indicative of differences in concentration-time profiles) [F(7,75)=50.18, $P<0.001$]. Administration of CEPEA gave significantly higher PEA levels in brain than did an equimolar dose of PEA at 15,30,60,90 and 120 min ($P<0.05$), and an opposite effect was seen at 5 min ($P<0.001$). In liver, equivalent analyses indicated a significant difference between effects of drug treatments [F(1,80)=338.51, $P<0.001$] and of time [F(7,80)=83.05, $P<0.001$] and a significant interaction between drug treatment and time [F(7,80)=42.66, $P<0.001$]. Injection of CEPEA produced significantly higher PEA levels in liver from 5-180 min ($P<0.001$) than did an equivalent dose of PEA. In blood, there was a significant difference in effects of drug treatments [F(1,80)=5.33, $P<0.025$), a significant effect of time [F(7,80)=21.54, $P<0.001$) and a significant interaction between drug treatment and time [F(7,80)=14.83, $P<0.001$]. Administration of CEPEA resulted in significantly higher PEA levels in blood than did an equimolar dose of PEA at 15,30,60 and 90 min ($P<0.05$) whereas at 5 min PEA administration provided higher levels than CEPEA treatment ($P<0.001$). These statistical significance tests were amply reflected in sustained elevations of PEA levels and/or decreased rate of elimination of the PEA formed from CEPEA in comparison to levels seen after treatment with PEA itself. Comparing the overall availability of PEA in brain, blood and liver after administration of an equimolar dose of PEA and CEPEA, CEPEA caused significantly more PEA available in liver, but not in brain or blood. However, the pattern of the increase in PEA levels in brain and blood was much different after CEPEA administration, with more consistent, relatively sustained elevations being attained after CEPEA than after PEA.

Both the _in_ _vitro_ and _in_ _vivo_ MAO inhibition data (Table 13) indicated CEPEA to be a weak MAO inhibitor, and a contribution of MAO inhibition to the elevations of PEA levels could be ruled out. The data obtained indicate that CEPEA undergoes metabolic N-dealkylation to yield PEA, thus acting as a prodrug. Such a dealkylation reaction for several N-alkylated phenylethylamines has been shown to be mediated by the cytochrome P-450 enzyme system (Duncan et al., 1985), although dealkylation by MAO cannot be ruled out for certain. Interesting differences were observed in the amount of PEA formed from CEPEA in the three tissues studied. Assuming the ratio of the AUC of PEA formed from CEPEA to that of CEPEA as an index of the extent of N-dealkylation in each tissue, the percentage fractions were 6.3, 34.3 and 13.8 in brain, liver and blood respectively. Because of the limited metabolic capacity of blood, most of the PEA in blood is presumably due to PEA formed from CEPEA in liver.

Preliminary studies in which CEPEA was administered to rats at doses of 0.1, 0.2 and 0.4 mmol/kg (i.p.) (Table 9) clearly indicated a fairly linear N-dealkylation following acute administration. This would indicate the possibility of employing higher doses of CEPEA to elevate PEA levels without saturating metabolic enzymes.

The distribution of CEPEA into brain was fairly rapid and the concentration-time profiles in brain and liver were superimposable (Fig. 36), although the availability in liver was significantly higher than in brain. In both organs, following a rapid decline phase, CEPEA was eliminated slowly. Such a rapid distribution followed by slower elimination was observed earlier for 2-N-phenylethylaminealkanenitriles in a scintigraphic study in dog (Winstead et al., 1978).

A.3 Rat tissue levels of PEA and CPPEA after administration of CPPEA

Statistical analysis of PEA levels in brain (Figs. 33 and 37) after an equimolar dose of PEA and CPPEA indicated a significant difference between the effects of drug treatments (i.e., PEA and CPPEA) [F(1,80)=48.76, $P<0.001$], a significant effect of time [F(7,80)=62.04, $P<0.001$] and a significant interaction between drug treatment and time [F(7,80)=61.47, $P<0.001$] in brain. Administration of CPPEA provided significantly higher PEA levels than did an equimolar dose of PEA at 15, 30, 60, 90 and 180 min ($P<0.05$), whereas PEA treatment gave significantly higher levels than CPPEA treatment at 5 min ($P<0.001$). Equivalent analysis of PEA levels in blood (Figs. 34 and 37) indicated a significant difference between the effects of treatments [F(1,80)=20.01, $P<0.001$], a significant effect of time [F(7,80)=18.21, $P<0.001$] and a significant interaction between treatment and time [F(7,80)=17.74, $P<0.001$]. Injection of CPPEA resulted in significantly higher blood PEA levels than did an equimolar dose of PEA from 60-180 min ($P<0.05$) whereas PEA treatment gave higher PEA levels at 5 and 15 min than did CPPEA treatment ($P<0.01$). Similar analysis of PEA levels in liver (Figs. 35 and 37) indicated a significant difference between the effects of drug treatment [F(1,80)=20.12, $P<0.001$], a significant effect of time [F(7,80)=62.51, $P<0.001$] and a significant interaction between drug treatment and time [F(7,80)=12.67, $P<0.001$]. Administration of CPPEA gave significantly higher PEA levels in liver than did an equimolar dose of PEA only at 180 min, whereas PEA treatment gave higher values at 5 and 30 min ($P<0.01$). These differences were clearly demonstrated in terms of altered clearance of PEA formed from CPPEA in comparison to rapid elimination of PEA after PEA treatment itself.

Following the administration of CPPEA, PEA levels increased well above controls and these levels were sustained for longer times than seen after administration of PEA itself (Fig. 33-35 and 37), although the levels were not as high as those seen after the administration of CEPEA. In all three tissues studied, PEA levels decreased biexponentially and the half-lives of distribution (α) and elimination (β) were higher than those seen after an equimolar dose of PEA, indicating slower elimination due to the prodrug effect of CPPEA. From the overall availability point of view, CPPEA administration resulted in significantly lower availability than that from an equimolar dose of PEA in brain and blood whereas in liver there were no differences (Fig. 44). Assuming the ratio of AUCs of PEA to CPPEA as an index of _in vivo_ N-dealkylation, the percentage ratios in brain, liver and blood were 1.3, 2.7 and 4.3 respectively. In view of the limited metabolic capacity of the blood compartment the ratio in blood is of little significance and probably represents diffusion, primarily from liver. These data indicate that liver was nearly twice as active as brain in converting CPPEA to PEA and perhaps this contributes to the slow elimination of PEA from it in comparison to that in brain and blood (Table 10). When compared to CEPEA metabolism to PEA (see Tables 8 and 10), the extent of CPPEA's conversion to PEA was lower, suggesting that CPPEA might be undergoing other metabolic reactions besides N-dealkylation. A related compound, N-(3-chloropropyl)amphetamine (Pondinil®), was studied for its metabolic disposition (Nazarali _et al._, 1983). The presence of the chloropropyl group had been proposed to render this amphetamine analog resistant to _in vivo_ N-dealkylation, and it was reported to undergo predominantly aromatic hydroxylation (Caldwell, 1976). Nazarali _et al._ (1983)

reported that the amount of amphetamine formed after administration of Pondinil® was significantly lower than that after an equimolar (0.1 mmol/kg, i.p.) dose of N-(2-cyanoethyl)amphetamine. It seems that a similar situation exists with the corresponding analogs of PEA since the data in Fig. 44 indicate that CEPEA administration gave a higher AUC for PEA in brain, liver and blood ($P<0.05$) than did an equimolar dose of CPPEA.

Distribution of CPPEA between brain and liver (Table 10) indicated no preferential tissue localization despite significant differences in peak levels between these tissues, which may be due to lipophilicity of CPPEA. Pondinil® was reported to be highly lipophilic and less than 1% of an administered dose in humans and in rats was excreted unchanged, indicating extensive metabolism (Caldwell, 1976). Like Pondinil®, CPPEA might also be undergoing extensive metabolism; distribution studies in other tissues as well as in urine would shed further light on the extent of tissue localization and metabolism.

A.4 Rat tissue levels of PEA and PGPEA after administration of PGPEA

Statistical analysis of brain PEA levels (from 0-180 min) after administration of equimolar doses of PEA (Fig. 33) and PGPEA (Fig. 39) indicated no overall significant difference between the effects of drug treatment (i.e., PEA and PGPEA) [$F(1,74)=1.85$, $P>0.2$], but a significant effect of time [$F(7,74)=25.79$, $P<0.001$] and a significant interaction of drug treatment and time (indicative of differences in concentration-time profiles) [$F(7,74)=35.14$, $P<0.001$] were noted. The massive increase in PEA levels at 5 min after PEA injection was probably responsible for the lack of an overall significant drug treatment effect. Administration of PGPEA provided significantly higher PEA levels than did an equimolar dose of PEA from 15 min to 180 min ($P<0.001$), where as at 5 min PEA treatment gave higher levels than PGPEA treatment ($P<0.001$). Equivalent analysis of PEA levels in blood (Figs. 34 and 39) revealed a significant difference between the effects of drug treatments [$F(1,78)=7.38$, $P<0.01$], a significant effect of time [$F(7,78)=19.30$, $P<0.001$] and a significant interaction between drug treatment and time [$F(7,78)=14.87$, $P<0.001$]. Treatment with PGPEA provided significantly higher blood PEA levels than did an equimolar dose of PEA from 30 to 180 min ($P<0.01$) whereas at 5 min the opposite effect was observed ($P<0.001$). Similar analysis of PEA levels (Figs. 35 and 40) in liver indicated a significant difference between the effects of drug treatments [$F(1,76)=103.93$, $P<0.001$], a significant effect of time [$F(7,76)=15.74$, $P<0.001$] and a significant interaction between drug treatment and time [$F(7,76)=10.76$, $P<0.001$]. Administration of PGPEA provided significantly higher PEA levels than did an equimolar dose of PEA from 5-180 min ($P<0.001$). Peak levels of PEA in brain and blood after administration of PGPEA were significantly below those after an equimolar dose of PEA itself ($P<0.05$) but in liver PGPEA administration gave higher peak levels than those seen after PEA ($P<0.001$). Levels of PEA in brain, blood and liver 4 hr after PGPEA treatment were approximately 10-30 times control values in comparison to the rapid decline after PEA administration.

Administration of PGPEA resulted in marked increases in PEA levels in all three tissues studied and these levels were still well above control PEA values at 4 hr, the longest time period studied. The elevated levels in all tissues decreased biexponenitally. The values were similar in all tissues, whereas the β value in blood was higher than those in brain and liver, indicating slower elimination. Liver contained quantitatively higher levels of PEA than did brain (evidenced by a higher AUC) throughout the time period studied, indicating a greater degree of N-dealkylation in liver than in brain. Assuming the ratio of the AUC of PEA to PGPEA as an index of _in vivo_ N-dealkylation, the % fractions in brain and liver were 26% and 35% respectively. Administration of PGPEA provided a greater amount of PEA available to brain and liver than did an equimolar dose of PEA and there were no overall differences between these treatments in blood. Besides elevating the levels of PEA, PGPEA administration sustained them more favourably over a long period of time, i.e. PEA levels showed more consistent and prolonged increases.

Several N-propargyl analogs derived from BZA (Swett _et al._, 1965) and amphetamine (Knoll _et al._, 1965, 1978) were demonstrated to be potent MAO inhibitors. Mechanistic studies revealed that these propargylamines act as suicidal substrates for the MAO enzyme, causing irreversible inactivation by interacting with cysteinyl flavin adenine-dinucleotide (cysteinyl-FAD), the prosthetic group for MAO (Singer, 1971; Salach _et al._, 1977; Fowler _et al._, 1982). Propargylamines are also known to inactivate non-flavin-dependent MAO (Rando and Mairena, 1974). Williams and Lawson (1974, 1975) studied the MAO-inhibiting properties of PGPEA and found it to be weak inhibitor of MAO. Even though the MAO-inhibiting properties of PGPEA are known, its inhibitory activity against MAO-A and -B and its ability to influence the levels of biogenic amines have never been investigated. The propargyl group is metabolically labile, as evidenced by the _in vivo_ formation of amphetamine and methamphetamine following the administration of deprenyl (N-methyl,N-propargylamphetamine) (Reynolds _et al._, 1978; Karoum _et al._, 1978; Philips, 1981; Coutts _et al._, 1981; Karoum _et al._, 1982) and benzylamine and N-methylbenzylamine from PAR (N-methyl,N-propargylbenzylamine) (Durden _et al._, 1975; Pirisino _et al._, 1978; Coutts _et al._, 1981; Weli and Lindeke, 1985, 1986). Thus PGPEA contains the necessary functional groups to render it an MAO inhibitor as well as a prodrug of PEA.

During the course of the present investigation, MAO inhibitory activity of PGPEA against MAO-A and -B was determined using specific substrates (see Table 14) and the results indicated relatively selective MAO-B inhibition after PGPEA administration. _In vivo_ N-dealkylation (depropargylation) as well as MAO-B inhibition may both contribute to the marked elevations in PEA levels. Lungs contain a mechanism for active uptake of PEA by MAO-B (Ben-Harare and Bakhle, 1980). The presence of quantitatively higher MAO activity in liver (Gorkin, 1983) as well as the existence of an uptake mechanism of PEA by lungs would probably mean that in untreated animals the amount of PEA formed in liver and then entering the brain via blood circulation would be minimal. Because of the MAO-B inhibition observed _in vivo_ after PGPEA treatment, it is possible that at least a portion of the PEA formed from PGPEA in liver might add to that already formed in brain.

An examination of the distribution of PGPEA revealed some interesting differences (Table 11). Liver contained quantitatively more PGPEA than brain, thus indicating peripheral tissue localization and relatively poor blood-brain barrier-penetrating abilities. The physicochemical properties of PGPEA might contribute to these differences. The pKa value of PEA is reported to be 9.64 (Williams, 1974) whereas that of PGPEA is 7.47 (Williams and Lawson, 1975); thus under physiological conditions, less than 1% of PEA would remain unionized whereas nearly 50% of PGPEA should remain unionized. This factor should improve tissue distribution characteristics of PGPEA relative to PEA, but the poor hydrocarbon-to-water partition coefficient (Williams and Lawson, 1975) (indicative of relative lipophilicity) of PGPEA relative to PEA appears to limit its penetration across the blood-brain barrier.

A.5 Rat tissue levels of PEA and PGPEA after administration of DPGPEA

Statistical analysis of brain PEA levels (0-180 min) following administration of equimolar doses of PEA (Fig. 33) and DPGPEA (Fig. 42) indicated a significant difference between effects of the drug treatments [F(1,72)=5.98, P<0.025], a significant effect of time [F(7,72)=44.63, P<0.001] and a significant interaction between drug treatment and time (indicative of differences in concentration-time profiles) [F(7,72)=49.74, P<0.001]. Treatment with DPGPEA provided significantly higher PEA levels than did an equimolar dose of PEA from 15-180 min (P<0.01) whereas at 5 min PEA administration gave higher peak levels than DPGPEA treatment (P<0.001). Similar analysis of PEA levels in blood (Figs. 34 and 42) indicated a significant difference between effects of drug treatments [F(1,73)=11.17, P<0.001], a significant effect of time [F(7,73)=14.83, P<0.001] and a significant interaction between drug treatment and time [F(7,72)=14.77, P<0.001]. Levels of PEA after DPGPEA administration were higher than after PEA treatment from 60-180 min (P<0.01) whereas at 5 min PEA treatment gave higher levels

(P<0.001). In liver, statistical analysis of PEA (Figs. 35 and 42) revealed a significant difference between effects of drug treatment [F(1,71)=162.98, P<0.001], a significant effect of time [F(7,71)=12.45, P<0.001] and a significant interaction between drug treatment and time [F(7,71)=10.67, P<0.001]. Administration of DPGPEA gave significantly higher PEA levels from 15-180 min (P<0.01) than did an equimolar dose of PEA.

Brain, blood and liver levels of PEA (Fig. 42) were increased well above the control PEA levels and these levels were sustained for up to 4 hr (the longest time interval studied). These changes in PEA levels were in contrast to the transient increase in PEA levels followed by rapid elimination after PEA administration itself. Administration of DPGPEA also prolonged the elimination of PEA from these tissues and caused significantly greater PEA available overall in brain and liver but not in blood compared to the situation after injection of PEA itself (Fig. 44).

As DPGPEA contains no derivatizable functional groups, attempts were made to quantitate DPGPEA by GC-NPD, but the sensitivity of DPGPEAy for this type of detector was found to be too low to enable a complete pharmacokinetic study to be performed. As a recourse, the levels of the major dealkylated metabolite of DPGPEA, namely PGPEA, were measured in the hope that these levels would reflect the levels of its precursor. Considering the ratio of the AUC of PEA to PGPEA as an index of N-dealkylation, nearly 30% of available PGPEA was metabolized to PEA in liver, whereas in brain the value was only 16%, indicating that liver was metabolically more active than brain. Liver also contained quantitatively more PGPEA than brain, suggesting that a significant amount of

DPGPEA is localized in the peripheral tissue compartment (Table 12). As with PGPEA, the physicochemical properties of DPGPEA might influence its peripheral localization. Compared to PEA, DPGPEA would expect to be weaker base because of the electron-withdrawing abilities of the propargyl groups. Until the present study, DPGPEA had neither been synthesized nor its physicochemical properties characterized. A related compound, N,N-dipropargylbenzylamine, was reported to have a pKa of 4.6 (Martin et al., 1975). Assuming the pKa of DPGPEA to be in the same range, nearly 99% of DPGPEA would be expected to remain unionized under physiological conditions and this should facilitate its passive diffusion across lipoidal membranes. But PGPEA, the metabolite of DPGPEA, was shown to have poor lipid solubility (Williams and Lawson, 1975); if DPGPEA also exhibits poor lipid solubility this would reduce its penetration across the blood-brain barrier and would mean that the levels of PGPEA observed in brain and liver are probably mainly due to N-dealkylation of DPGPEA in respective tissues. The monoalkylated PGPEA can further undergo N-dealkylation to form PEA, which, because of its lipophilicity and because of inhibition of MAO-B in these treated animals, can easily cross the blood-brain barrier, diminishing the effects of first-pass metabolism in liver and lung (see Section IV A.4).

The dialkylated drug DPGPEA represents an unique compound which acts as a double prodrug i.e., a prodrug of PEA and of an MAO inhibitor (PGPEA) which itself is a prodrug of PEA. In the present study, DPGPEA was also shown to be a weak but fairly selective MAO-B inhibitor in its own right, and these properties might contribute to the relatively sustained elevations of PEA at later time intervals compared to those seen after PGPEA (see Figs. 39, 40 and 42).

Statistical analysis of brain PEA levels (0-240 min) after an equimolar dose of PGPEA and DPGPEA (Figs. 39 and 42)indicated a significant difference between the effects of treatment (i.e., PGPEA and DPGPEA) [F(1,82)=19.97, P<0.001], a significant effect of time [F(8,82)=17.43, P<0.001] and a significant interaction between drug treatment and time [F(8,82)=8.65, P<0.001]. Similar analysis in blood (Figs. 39 and 42) revealed a significant difference between the effects of drug treatments [F(1,83)=20.43, P<0.001], a significant effect of time [F(8,83)=16.01, P<0.001] and a significant interaction between drug treatment and time [F(8,83)=4.88, P<0.001]. Similar analysis in liver (Figs. 40 and 42) indicated a significant difference between the effect of drug treatments [F(8,82)=12.84, P<0.001], a significant effect of time [F(8,82)=16.93, P<0.001] and a significant interaction between drug treatment and time [F(8,82)=6.93, P<0.001]. These differences were reflected in significantly higher peak levels and significantly greater availability (AUC) of PEA in brain, blood and liver after PGPEA administration than after an equimolar dose of DPGPEA. Administration of DPGPEA resulted in a more gradual increase in PEA levels than that seen after PGPEA injection.

The overall availability of PGPEA after administration of PGPEA and DPGPEA was not different in any tissue (P>0.05) (Fig. 45). Statistical analysis of brain PGPEA levels (0-180 min) after administration of an equimolar dose of PGPEA and DPGPEA (Fig. 41 and 43) revealed no significant difference between the effects of treatments [F(1,77)=3.93, P>0.05], a significant effect of time [F(7,77)=19.18, P<0.001] and no significant interaction between drug treatments and time [F(7,77)=2.21, P>0.05). Equivalent analyses in blood indicated no significant difference between effects of drug treatments [$F(1,72)=2.98$, $P>0.05$], but a significant effect of time [$F(7,72)=11.71$, $P<0.001$] and a significant interaction between drug treatments and time [$F(7,72)=2.80$, $P<0.05$). Similar analysis in liver indicated a significant difference between effects of drug treatments [$F(1,77)=7.18$, $P<0.01$], a significant effect of time [$F(7,77)=22.62$, $P<0.001$] and a significant interaction between drug treatments and time [$F(7,77)=6.42$, $P<0.001$].

B. In Vivo and In Vitro Inhibition of MAO by PGPEA and DPGPEA

In vitro MAO inhibitory data on PGPEA and DPGPEA (Table 16) indicated that PGPEA was more potent than DPGPEA at inhibiting MAO-A and MAO-B, while DPGPEA was more selective than PGPEA at inhibiting MAO-B relative to MAO-A. Compared to clorgyline, deprenyl, TCP and PAR (with reported IC$_{50}$ values in the nanomolar range), PGPEA and DPGPEA are weaker inhibitors *in vitro*. The *in vivo* inhibitory profile of PGPEA and DPGPEA indicated that they caused MAO-B inhibition fairly selectively in brain, while in liver the selectivity was more pronounced (Tables 14 and 15) in that MAO-A inhibition after PGPEA and DPGPEA in brain ranged from 20-40% while in liver it was 1-6%, with nearly 60-70% MAO-B inhibition seen in both tissues. The reasons for such tissue-dependent inhibitor selectivity are not clear. Knoll *et al.* (1978) observed that several (-)-deprenyl-derived inhibitors caused greater MAO-A inhibition in brain than in liver and this phenomenon appeared to be dose-dependent in that (-)-deprenyl and U-1424 [N-methyl, N-propargyl-(2-furanyl-methyl)ethylamine.HCl] exhibited such a selectivity at a dose of 10 mg/kg (i.p.) whereas J-508 [N-methyl-N-propargyl-(1-indanyl)amine.HCl] exhibited similar tissue-dependent MAO inhibition at a dose of 1.0 mg/kg (i.p.). Route of administration also appeared to influence tissue-dependent inhibition of MAO. Waldmeier et al. (1981) reported that irreversible inhibitors caused greater MAO inhibition in liver upon oral administration, whereas greater inhibition was observed in brain upon subcutaneous (s.c.) administration. This phenomenon was explained in terms of improved availability of inhibitor to liver upon oral administration and improved availability to brain upon s.c. administration. Significantly greater availability of inhibitor (PGPEA and DPGPEA) to liver than to brain would appear to negate such an explanation in the present case. Although literature reports indicate greater turnover of MAO in liver than in brain (Felner and Waldmeier, 1979), there is no evidence to indicate that MAO-A and -B differ from one another in their turnover rates. Knoll et al. (1978) reported that TZ-650 [N-methyl-N-propargyl-(2-phenyl)ethylamine.HCl, a potential prodrug of PEA] in in vitro studies was 5 times more potent at inhibiting PEA metabolism in liver than in brain. During the present investigation MPGPEA (TZ-650) was examined for its in vivo inhibitory activity, and the results indicate slightly higher MAO-B inhibition in liver than in brain at a dose of 0.1 mmol/kg (i.p.); however, its selectivity was much less marked than that of PGPEA and DPGPEA (Tables 14 and 15). If PGPEA and DPGPEA are also more potent at inhibiting PEA deamination in liver than in brain, this might, in part, contribute to the observed selectivity in liver. Further studies are required to elucidate the mechanisms underlying tissue-dependent MAO-A and -B inhibition. The irreversible MAO inhibitor TCP and the MAO-B selective inhibitor PAR at a dose of 0.1 mmol/kg (i.p.) almost completely inhibited both MAO-A and -B in brain and liver

(Table 14). It would be interesting to study whether PGPEA and DPGPEA exhibit MAO-B selectivity in a dose-dependent manner and upon chronic administration. Despite the differences in *in vitro* MAO inhibitory profiles of PGPEA and DPGPEA, the *in vivo* profiles indicate similarities between these inhibitors; this may be due to the metabolic interrelationships existing between these two compounds.

C. Levels of T in Brain and Liver Following the Administration of PGPEA and DPGPEA

There were significant increases in brain and liver T levels compared to the control levels in these tissues following PGPEA treatment, but there were no differences between brain and liver levels in the treated animals. This was in contrast to marked differences in the levels of PEA in brain and liver after injection of PGPEA. In quantitative terms, increases in T levels were not as high as those seen with PEA levels. Following DPGPEA treatment, T levels could not be detected in brain and liver. This cannot be explained in terms of differences in *in vivo* MAO inhibitory potencies between PGPEA and DPGPEA, and further studies are required to examine the differences between PGPEA and DPGPEA.

D. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA in Rat Brain Following the Administration of PEA and its Prodrugs

The interactions of elevated levels of PEA and its prodrugs with putative neurotransmitter amines have been examined by studying the levels of NA, DA, 5-HT and some acid metabolites of the amines. In order to control possible changes in these neurochemicals during storage or due to circadian variations, a saline-treated group was included in each treatment and the samples were analysed within 3-4 days after collection. A set of standards was run with each analysis and the standard curves were linear over the concentration range in which the neurochemicals were quantitated in brain samples.

Like TA, PEA is an indirect acting sympathomimetic amine, i.e., its peripheral actions are thought to be mediated, at least in part, by the liberation of endogenous NA. Because of its lipophilicity, PEA can cross the blood-brain barrier easily and produce central effects. Holtz et al. (1947) observed signs of central excitation in guinea pigs after high doses of PEA (50-150 mg/kg). After pretreatment of rats with an MAO inhibitor, behavioral effects of PEA (10-40 mg/kg) were difficult to distinguish from those of (+)-amphetamine (Mantegazza and Riva, 1963). Jonsson et al. (1966) observed depletion of NA to nearly 50% of control levels within 30 min after administration of PEA at a dose of 100 mg/kg (i.m.); by 60 min NA levels decreased to nearly 35% of control values. A second injection of the same dose did not lower the NA levels any further. In a group of rats receiving 50 mg/kg (i.m.) of PEA every 15 min (total 400 mg/kg), NA levels in brain decreased to nearly 13% of control values by 120 min (Jonsson et al., 1966), and subcellular distribution studies indicated preferential depletion from the particulate fraction. Fuxe et al. (1967) extended these studies by employing histochemical techniques to visualize catecholamine-containing neurons; they observed depletion of both NA and DA from their nerve terminals by PEA. Administration of PEA at a dose of 100 mg/kg (i.m.) caused nearly 50% depletion of NA while DA levels increased nearly 20% above controls at 60 min after administration. Pretreatment of rats with nialamide (an MAO inhibitor) followed by PEA injection (100 mg/kg, i.m.) decreased brain NA levels to nearly 35% of levels in brains of rats treated with nialamide only, without significantly affecting DA levels. Multiple administration regimens of PEA (8x50 mg/kg, i.m., every 15 min or 4x100 mg/kg, i.m., every 15 min for 2 hr) caused almost 65% and 25% depletions in NA and DA brain levels respectively. Jackson and Smythe (1973) reported significant depletions in whole brain NA and DA but not of 5-HT, 60 min following PEA administration (100 mg/kg, i.p.), while regional analysis indicated significant depletions of 5-HT levels in midbrain and cortex. These authors also reported depletion of NA, DA and 5-HT from mouse brain by PEA, and Jackson (1971) observed NA depletion in guinea pig brain following PEA injection (100 or 200 mg/kg, i.p.). Jackson and Smythe (1974) observed that PEA increased NA turnover without affecting DA turnover. Karoum et al. (1982) found significant depletions of NA levels in hypothalamus 20 min after a dose of PEA of 20 mg/kg (i.v.); DA levels also decreased, while DOPAC levels were increased significantly above controls at 7.5 min and decreased below controls by 20 min. In caudate nucleus, NA levels were significantly higher than control levels by 20 min, while DA levels decreased below controls at 7.5 min and increased above control levels at 20 min; DOPAC levels exhibited a diametrically opposite pattern to that seen for DA. Sloviter et al. (1980) reported decreases in NA levels in whole brain to nearly 82% of controls levels at 10 min following an acute dose of PEA (80 mg/kg, i.p.); this dose was without effect on DA and 5-HT concentrations. Chronic administration of PEA (100 mg/kg, i.p. twice daily for 10 days) increased levels of both NA and MHPG (the principal metabolite of NA) in hypothalamus (Karoum et al., 1982). In vitro synaptosomal data of Raiteri et al. (1977) and recent electrophysiological data of Lundberg et al. (1986) indicate catecholamine-releasing properties of PEA. From a survey of the literature it is evident that neurochemical effects of PEA on NA appear to be dependent upon dose, frequency of administration, pretreatment and time after administration as well as on region of the brain studied. In the earlier studies cited above, the doses employed were about 5-10 times higher than those in the present study and the levels of NA were often measured at a single time point, unlike the situation in the present investigation where levels were measured at several different times. The decreases in NA levels seen in the present investigation appear to be proportional to the dose administered in the light of earlier data. These decreases were observed at 30 and 60 min after administration of PEA (although there was a trend indicating a decrease in NA levels at 15 min), by which time PEA levels in whole brain were about 4-5 times control levels. This points out that large increases in PEA levels at initial times did not produce immediate marked changes in NA levels.

Edwards and Antelman (1978) observed that striatal HVA levels (an index of activity of DA neurons in striatum) decreased below controls at 5 min following injection of a dose of PEA of 100 mg/kg (i.p.) and increased above control levels by 60 min, whereas DA levels increased significantly above controls at 5 min and decreased to control levels thereafter. Studies by Raiteri et al. (1977), Baker et al. (1976, 1978), Baker and Yasensky (1981), Roberts (1981), Dyck (1983, 1984), Philips and Robson (1983), Nielsen et al. (1983), and McQuade and Wood (1983) have demonstrated the DA-releasing properties of PEA. McQuade and Wood (1983) observed increases in DA levels in caudate nucleus 20 min after injection of PEA (12.5 mg/kg, s.c.) and at 15 min after a 25 mg/kg dose; these increases were associated with increases in DOPAC and HVA levels, indicating stimulation of DA synthesis. Smaller doses of PEA increase DA release without affecting its reuptake, whereas higher doses of PEA not only increase release but also inhibit reuptake (Roberts and Patrick, 1979; Nielsen _et al._, 1983). Increased release without reuptake blockade relieves tyrosine hydroxylase enzyme from feedback inhibition, leading to increased DA synthesis, whereas increased release coupled with reuptake blockade activates presynaptic receptor-mediated DA synthesis inhibition (Roberts, 1981; Roberts and Patrick, 1979; Nielsen _et al._, 1983). Roberts and Patrick (1979) suggested that increased DA synthesis after low doses of PEA might also be due to increased _p_-tyrosine uptake. Increased DA and NA levels were noted in the cerebrospinal fluid of awake primates following PEA administration (Perlow _et al._, 1980). In the present investigation, DA and HVA levels remained unchanged, whereas DOPAC levels decreased initially and then increased to control levels. Despite the marked increase in PEA levels at initial times, PEA levels decreased rapidly, hence the neurochemical effects on the dopaminergic systems appear to be short-lived.

It has been reported that PEA is a weak inhibitor of 5-HT uptake in human platelets (Richter and Smith, 1974) as well as in rabbit platelets (Tuomisto _et al._, 1974). Jackson and Smythe (1973) reported decreases in 5-HT levels in rat midbrain, cortex and in mouse brain after injection of PEA (100 mg/kg, i.p.). In rat striatal slices, Dyck (1984) showed significant release of tritiated 5-HT by PEA. Anden _et al._

(1974) reported that some hallucinogenic phenylethylamines decrease 5-HT synthesis through receptor interaction. The initial behavioral effects of PEA seem to be more serotoninergic than dopaminergic in nature (Dourish, 1981, 1982), although PEA was a weaker inhibitor of 5-HT uptake than of DA (Raiteri *et al.*, 1977) *in vitro*. Acute (Sloviter *et al.*, 1980; Dourish, 1981) and chronic (Dourish, 1981) administration of PEA resulted in a hyperreactivity syndrome that seem to be mediated predominantly by 5-HT mechanisms. Systemic injection of PEA (75 mg/kg, i.p., 1 hr before sacrifice) was reported to increase the concentration of brain tryptophan and 5-HT synthesis in some brain regions (Snodgrass and Uretsky, 1978), and chronic administration (75 mg/kg, i.p., once daily for 5 days, 12 hr before sacrifice) did not alter 5-HT synthesis. At high concentrations PEA is known to condense with pyridoxal at physiological pH to form an aldimine (Schiff base) which inhibits tryptophan decarboxylase, an enzyme responsible for the biosynthesis of 5-HT (Loo *et al.*, 1978). With the transient increases in brain PEA levels seen after PEA administration in the present study, inhibition of 5-HT synthesis is likely *not* an important factor. Decreases in 5-HIAA levels initially would suggest decreased deamination, possibly because of reduced reuptake of released 5-HT.

Administration of CEPEA caused some changes in levels of neurotransmitters and their metabolites (see Figs. 47-52) . Decreases in NA, DA and 5-HT levels occurred during 30-120 min and at these times PEA levels in brain were significantly elevated above control levels. In contrast to transient changes in neurotransmitter amines and their metabolites seen after administration of PEA alone, effects after CEPEA administration were more pronounced and long-lasting. Fuxe *et al.*

(1967) observed marked depletion in rat brain NA levels after PEA injection to MAO inhibitor-pretreated rats in comparison to an MAO inhibitor-pretreated control group. This would suggest that sustained elevation of PEA levels lead to marked NA depletion. In contrast to the short-lived increases in brain PEA levels seen after PEA injection, CEPEA administration caused sustained elevation of PEA levels. Statistical analysis of NA levels after PEA and CEPEA administration at different times (5-180 min) indicated a significant difference between the effects of drug treatments [F(1,80)=16.95, P<0.001] and a significant effect of time [F(6,80)=4.96, P<0.001], but no significant overall interaction between drug treatment and time [F(6,80)=0.64, P>0.2) was noted.

Analysis of variance of DA levels after PEA and CEPEA administration indicated a significant difference between effects of drug treatments [F(1,80)=21.98, P<0.001], a significant effect of time [F(6,80)=3.63, P<0.01] and a significant interaction between drug treatment and time [F(6,80)=2.26, P<0.025]. Equivalent analysis of DOPAC levels after PEA and CEPEA administration indicated no significant differences between effects of drug treatments [F(1,88)=0.27, P>0.2], but a significant effect of time [F(6,68)=2.76, P<0.025] and a significant interaction between time and drug treatment [F(6,88)=3.49, P<0.01]. In the case of HVA levels after PEA and CEPEA administration, there were significant differences between effects of drug treatments [F(1,85)=14.42, P<0.001], a significant effect of time [F(6,85)=3.53, P<0.005] but no interaction between drug treatment and time [F(6,85)=1.39, P>0.2]. Changes in DA levels indicate that CEPEA administration might be mimicking large dose PEA administration. Increased HVA levels, without changes in DOPAC levels, coupled to persistent decreases in DA levels, would suggest DA release, uptake blockade, metabolism of released DA via the COMT enzyme system and possibly DA synthesis inhibition. Defining DA turnover as the ratio of the sum of DOPAC and HVA (% controls) to DA (% controls) (defined baseline value would be 2), PEA administration was observed to decrease this ratio to slightly below 2 at initial times (5-30 min). Administration of CEPEA caused this ratio to increase to between 2.2-2.6 up to 120 min, indicating increased DA turnover.

Analysis of variance of 5-HT levels after PEA and CEPEA administration indicated a significant difference between the effects of drug treatments [$F(1,90)=8.85$, $P<0.005$], but no significant effect of time [$F(6,90)=2.11$, $P>0.05$] and no significant interaction between drug treatment and time [$F(1,90)=1.72$, $P>0.2$] were noted. Statistical analysis of 5-HIAA levels after PEA and CEPEA administration indicated a significant difference between effects of drug treatments [$F(1,86)=13.14$, $P<0.001$], a significant effect of time [$F(6,86)=5.15$, $P<0.001$] and a significant interaction between drug treatment and time [$F(6,86)=2.73$, $P<0.05$]. Decreases in 5-HT levels with relatively smaller increases in 5-HIAA levels following CEPEA administration would suggest increased 5-HT turnover. In contrast to minor changes in 5-HT turnover [defined as ratio of 5-HIAA to 5-HT (% control)] after PEA treatment (values from 0.9 to 1.15 at 5-180 min), CEPEA treatment increased 5-HT turnover (values from 1 to 1.6 during 5-180 min).

The administration of the prodrug caused more pronounced decreases in NA, DA and 5-HT than did PEA, although in both cases the effects were relatively weak and short-lived. One has to consider the direct effect of the prodrug itself on these neurotransmitters and metabolites besides the effect of elevated PEA. A series of N-n-alkylated analogs of PEA has been shown to be active at inhibiting 5-HT uptake mechanisms (Suckling _et al._, 1985), and other N-alkylated analogs of phenylethylamines are known to affect the uptake and release of these biogenic amines (Koe, 1976; Baker _et al._, 1980a). Uptake and release experiments with CEPEA alone and in combination with PEA would indicate the ability of CEPEA to affect the neurotransmitters as well as any synergistic effect PEA might have on CEPEA.

The changes in levels of NA, DA, 5-HT and the acid metabolites HVA, DOPAC and 5-HIAA after the administration of CPPEA (Figs. 53-55) were more marked than those observed after CEPEA and PEA administrations (Figs. 47-52). Analysis of NA levels (5-180 min) after administration of equimolar doses of PEA or CPPEA revealed a significant difference between the effects of drug treatments [F(1,62)=201.77, P<0.001], a significant effect of time [F(6,62)=15.72, P<0.001] and a significant interaction between drug treatment and time [F(6,62)=7.32, P<0.001]. In the case of DA levels, there was a significant difference between the effects of drug treatments [F(1,68)=50.99, P<0.001], and a significant effect of time [F(6,68)=8.67, P<0.001] but no significant interaction between drug treatments and time was noted [F(6,68)=1.92, P>0.1]. Analysis of HVA levels indicated no significant difference between effects of drug treatments [F(1,61)=0.17, P>0.2], but a significant effect of time [F(6,61)=5.93, P<0.001] and a significant interaction between drug treatments and time [F(6,61)=2.45, P<0.05] were noted . The results with DOPAC levels revealed no significant difference between effects of drug treatments [F(1,62)=0.65, P>0.2], but a significant effect of time [F(6,62)=3.14, P<0.025] and a significant interaction between time and drug treatments [F(6,62)=4.9, P<0.001] were noted. Statistical analysis of 5-HT concentrations revealed a significant difference between the effects of drug treatment [F(1,67)=14.13, P<0.001], a significant effect of time [F(6,67)=4.94, P<0.001] and a significant interaction between drug treatments and time [F(6,67)=3.05, P<0.025]. With 5-HIAA levels there was a significant difference between the effects of treatment [F(1,68)=9.93, P<0.005], and no significant effect of time [F(6,68)= 2.09, P>0.05], but a significant interaction between drug treatments and time [F(6,68)=7.31, P<0.001] was noted. Injection of CPPEA markedly depleted NA, with smaller but significant decreases observed for DA and 5-HT. Calculations revealed that CPPEA administration markedly increased DA turnover up to 90 min (from a baseline value of 2 to 3.3) and decreased it slightly below the baseline value at 120 min and 180 min. Similar analysis for 5-HT (ratio of 5-HIAA to 5-HT) also indicated increased 5-HT turnover after CPPEA up to 120 min (from a baseline value of 1 to 1.6) after drug administration.

Levels of PEA in brain after CPPEA administration were significantly higher than control levels, but these increases were not as high as those seen after administration of CEPEA. The effects of CPPEA administration on the neurotransmitter amines and metabolites were much more pronounced than those seen after CEPEA. This suggests that the effects of CPPEA may be more predominant than those of altered PEA levels in affecting these neurotransmitters. It is a lipophilic compound, and its levels (AUC) in brain were highest among the four prodrugs examined (Fig. 44); this might influence the uptake and release of neurotransmitters as other N-alkylated phenylethylamines have been reported to do (Koe, 1976; Baker _et_ _al._, 1980a; Suckling _et_ _al._, 1985).

Following PGPEA administration there were marked decreases in brain

NA and DA levels without significant changes in 5-HT levels, in contrast to the changes seen after PEA treatment (see Figs. 56-61 and 47-52). Comparisons of NA levels after administration of an equimolar dose of PEA and PGPEA revealed a significant difference between the effects of drug treatments [F(1,75)=41.90, P<0.001], a significant effect of time [F(6,75)=7.02, P<0.001] but no significant interaction between treatments and time [F(6,75)=1.73, P>0.2]. Analysis of DA levels under the same conditions indicated a significant difference between effects of drug treatments [F(1,80)=50.75, P<0.001], a significant effect of time [F(6,80)=5,81, P<0.001] but no significant interaction between drug treatment and time [F(6,80)=1.67, P>0.1]. In the case of DOPAC levels, there was no significant difference between effects of drug treatment [F(1,84)=3.25, P>0.1], but a significant effect of time [F(6,84)=2.27, P<0.05] and a significant interaction between drug treatment and time [F(6,84)=3.10, P<0.01] were noted. Analysis of HVA levels indicated no significant difference between effects of drug treatment [F(1,72)=0.34, P>0.2], but a significant effect of time [F(6,72)=4.09, P<0.005] and a significant interaction between drug treatment and time [F(6,72)=2.31, P<0.05]. Calculations of DA turnover indicated an increase in DA turnover (in the range of 2.5-3.1 compared to baseline value of 2.0) at initial times. Changes in DA, DOPAC and HVA levels (Figs. 57-59) suggest a release of DA and inhibition of reuptake. Analysis of 5-HT levels indicated no significant differences between the effects of drug treatment [F(1,84)=1.63, P>0.1], no significant effect of time [F(6,84)=1.71, P>0.1] and also no significant interaction between drug treatment and time [F(6,84)=1.11, P>0.1]. Statistical analysis of 5-HIAA levels indicated a significant difference between effects of drug treatments [F(1,87)=25.17, P<0.001], a significant effect of time [F(6,87)=5.54, P<0.001] and a significant interaction between drug treatment and time [F(6,87)=8.62, P<0.001].

The observations of Fuxe _et_ _al._ (1967) mentioned earlier suggested that pharmacological manipulations tending to prolong elevated PEA levels might result in marked decreases in catecholamine levels. Administration of PGPEA not only elevates PEA levels but also sustains them and concomitantly causes inhibition of MAO-A and -B to varying degrees, and thus in effect mimics the conditions used in the study of Fuxe _et al._ (1967). Administration of PGPEA caused nearly 20-30% inhibition of MAO-A and one would expect small decreases in 5-HIAA levels but not increases (Fig. 61). The changes in 5-HT and 5-HIAA levels observed would suggest enhanced release of 5-HT without reuptake inhibition and imply predominance of uptake effects over inhibition of MAO-A. Besides the interactions of PEA with NA, DA and 5-HT discussed earlier, one has to consider the possible effect of PGPEA itself on uptake and release of these amines. Lai _et_ _al._ (1980) demonstrated that the selective MAO inhibitors clorgyline and deprenyl, which are N-propargyl compounds respectively, in addition to inhibiting MAO also affect uptake of NA, DA and 5-HT in rat brain synaptosomes. Baker _et_ _al._ (1978, 1980b) also demonstrated the ability of irreversible, nonselective inhibitors such as PLZ, TCP and pheniprazine to affect uptake and release of NA, DA and 5-HT. Recently Dyck (1983, 1984) reported the ability of PLZ to release DA and 5-HT from rat striatum. It is equally conceivable that PGPEA can exert such actions on these biogenic amines in its own right.

Changes in levels of neurotransmitters and their metabolites after administration of DPGPEA were fairly similar to those seen after administration of PGPEA. Injection of DPGPEA caused more sustained depletions in NA and DA (without affecting 5-HT levels) than those seen after an equimolar dose of PEA (see Figs. 62-67 and 47-52).

Statistical analysis of brain NA levels after administration of equimolar doses of PEA or DPGPEA revealed a significant difference between effects of drug treatments [F(1,74)=19.77, P<0.001], a significant effect of time [F(6,74)=6.29, P<0.001] but no significant interaction between drug treatment and time [F(6,74)=1.29, P>0.1]. Similar analysis for DA indicated a significant difference between effects of drug treatment [F(1,92)=80.96, P<0.001] and a significant effect of time [F(6,92)=2.40, P<0.05] but no significant interaction between drug treatment and time [F(6,72)=0.91, P>0.2]. For DOPAC, a significant difference between the effects of drug treatment [F(1,85)=37.97, P<0.001], a significant effect of time [F(6,85)=2.26, P<0.05] and a significant interaction between drug treatment and time [F(6,85)=3.42, P<0.01] were noted. With HVA, there was no significant difference between effects of drug treatments [F(1,72)=2.64, P>0.4], but a significant effect of time [F(6,72)=7.49, P<0.001] and a significant interaction between drug treatment and time [F(6,72)=4.48, P<0.001] were observed.

Calculations of DA turnover values indicated that DPGPEA administration increased the turnover value of DA from a baseline value of 2.0 to about 2.5-3.0 up to 60 min followed by gradual decrease below baseline value by 180 min. Persistent decreases in levels of DA and DOPAC levels from 5-180 min (Figs. 63-65) coupled with marked increases in HVA levels at initial times suggest increased release of DA with reuptake inhibition. Analysis of variance of 5-HT levels indicated a significant difference between effects of drug treatments [F(1,92)=5.33, P<0.025], a significant effect of time [F(6,92)=2.28, P<0.05], but no significant interaction between drug treatment and time [F(6,92)=0.39, P>0.2]. Individual comparisons of 5-HT levels at each time interval (5-180 min) between PEA- and DPGPEA-treated groups were not significant. From the data in Figs. 51 and 66 it is, however, apparent that the drug treatment effect is attributable to overall decreases in 5-HT levels in the DPGPEA-treated group relative to those seen in the PEA-treated group. Analysis of 5-HIAA levels revealed a significant difference between effects of drug treatments [F(1,97)=13.62, P<0.001], a significant effect of time [F(6,97)=6.38, P<0.001] and a significant interaction between drug treatment and time [F(6,97)=2.36, P<0.05]. These changes were reflected in higher increases (P<0.05) in 5-HIAA levels at 5 and 15 min after DPGPEA treatment in comparison to those seen after PEA injection. Administration of DPGPEA increased the turnover index of 5-HT (from a baseline value of 1.0) up to 120 min to a value in the range of 1.25-1.4.

Sustained elevations in brain PEA levels, MAO inhibition and possible abilities of DPGPEA to influence the uptake and release of NA, DA, 5-HT may be contributing to the neurochemical profile of DPGPEA. Because of metabolic interrelationships existing between DPGPEA and PGPEA, it is likely that levels of PGPEA may also influence to the overall pharmacodynamic and neurochemical profile.

E. Discussion of Pharmacokinetic and Neurochemical Effects of PEA and its Prodrugs

During the course of the present investigation pharmacokinetic and neurochemical properties of four potential PEA prodrugs, namely CEPEA, CPPEA, PGPEA and DPGPEA, have been examined and compared to those of PEA itself. Sensitive GC-ECD techniques have been developed to quantitate levels of PEA and its prodrugs in brain, blood and liver. Administration of PEA itself caused marked increases in tissue PEA levels, but these levels decreased rapidly, probably due to rapid deamination and/or distribution. These results indicate that PEA itself cannot be employed to elevate PEA levels. Repeated administration of PEA could theoretically be employed, but rapid fluctuations in tissue PEA levels would not reveal any meaningful information as to the possible role of PEA in the CNS. N-Alkylated prodrugs have been examined with a view that they would undergo *in vivo* conversion to PEA to elevate and sustain the levels of PEA selectively, i.e. without affecting other neurotransmitters or modulators. Administration of drugs inhibiting MAO could achieve this objective albeit nonspecifically, i.e., there would be increases in levels of several bioactive amines. All the N-alkylated analogs of PEA investigated were demonstrated to undergo *in vivo* N-dealkylation, generating PEA, although there were differences in the changes in PEA. *In vivo* MAO inhibition data indicated CEPEA to be a weak MAO inhibitor, whereas PGPEA and DPGPEA were found to be fairly selective MAO-B inhibitors. Although CPPEA was not examined for its *in vivo* inhibition, lack of structural features known to impart MAO inhibiting properties, and relatively smaller increases in PEA levels in comparison to ~~those~~ after administration of other prodrugs suggest a weaker

MAO inhibitory activity, if any. Administration of CEPEA resulted in gradual increases in brain PEA levels and these levels were sustained in comparison to the transient changes after treatment with PEA itself. Besides sustaining PEA levels, CEPEA treatment was shown to increase availability of PEA to liver. Administration of CPPEA caused moderate increases in PEA levels and these levels were sustained for longer times than seen after injection of PEA itself, indicating delayed clearance of PEA after CPPEA administration as well. Overall availability of PEA in brain was lower after CPPEA administration than after PEA administration, but this is somewhat misleading since a comparison of the actual time-response curves reveals that CPPEA produces smaller, but more sustained levels of PEA than does administration of PEA. Comparison of present experimental data with those of Nazarali _et al._ (1983) and Coutts _et al._ (1986b) suggests that there are differences between cyanoethyl and chloropropyl groups as alkyl groups with regard to _in vivo_ N-dealkylation. Metabolic studies in amphetamine analogs indicated that N-cyanoethylamphetamine is rapidly N-dealkylated to generate the parent amine, amphetamine, both in humans and in rats (Beckett and Shenoy, 1972, 1973; Coutts _et al._, 1986b); the possible influence of the electron-withdrawing ability of the cyanoethyl group in rapid metabolic conversion has been proposed (Beckett and Shenoy, 1972, 1973). N-(3-Chloropropy)-amphetamine (Pondinil®) is structurally related to N-cyanoethylamphetamine, and metabolic studies revealed very little _in vivo_ N-dealkylation (Calwell, 1976; Nazarali _et al._, 1983) and a preponderance of aromatic hydroxylation (Caldwell, 1976). Comparisons of conversions of CEPEA and CPPEA to PEA indicate that the cyanoethyl group was more labile than the chloropropyl group to metabolic N-dealkylation, similar to the situation with the N-alkylated amphetamines.

The propargyl analogs PGPEA and DPGPEA are interesting compounds in that they are weak but fairly selective MAO-B inhibitors as well as prodrugs of PEA. Both were shown to be more highly selective inhibitors of MAO-B in liver than in brain *in vivo*. The dipropargyl compound DPGPEA was found to be a prodrug of PGPEA, which in turn was shown to be a prodrug of PEA; thus DPGPEA represents a double prodrug of PEA. The fact that PGPEA and DPGPEA are MAO inhibitors *in vivo* raises the question as to the relative contributions of *in vivo* MAO inhibition and N-dealkylation to the increases in PEA levels. The observation that increasing PEA levels after PGPEA and DPGPEA were higher than those observed after administration of selective or nonselective MAO inhibitors (Boulton *et al.*, 1973; Philips and Boulton, 1979; Axelrod and Saavedra, 1974; Baker *et al.*, 1984a; Philips, 1984; Lauber and Waldmeier, 1984) would suggest that N-dealkylation may be predominating over inhibition of MAO. However, both effects are probably complementary to each other in elevating and sustaining PEA levels. These studies have also demonstrated that the N-propargyl group is metabolically labile and this extends earlier reports in which N-propargyl analogs of N-methylbenzylamine and N-methylamphetamine were shown to undergo *in vivo* N-dealkylation (Durden *et al.*, 1975; Pirisino *et al.*, 1978; Reynolds *et al.*, 1978; Philips, 1981; Coutts *et al.*, 1981; Karoum *et al.*, 1982; Weli and Lindeke, 1985, 1986).

Studies conducted so far with N-cyanoethyl and N-propargyl analogs of phenylalkylamines would suggest that these two groups represent a useful means of extending the levels of such amines in brain tissue.

Comparative availabilities (AUCs) of PEA after administration of

PEA and its prodrugs in blood, brain and liver are presented in Fig. 44. The ranking of treatments, in decreasing order of AUC of PEA in brain was PGPEA>DPGPEA>>CEPEA,PEA>CPPEA; in blood the ranking order was PEA,CEPEA,PGPEA>DPGPEA>CPPEA and in liver the ranking was PGPEA>DPGPEA> CEPEA>>CPPEA>PEA. Ideally a good prodrug should provide elevated levels and sustain them; this is as important as, or more important than, overall increased bioavailability. The N-cyanoethyl analog CEPEA (a weak MAO inhibitor) is a better candidate than CPPEA in providing such features. The dipropargyl analog DPGPEA (an MAO-inhibiting prodrug) elicits gradual, sustained elevations of PEA; PGPEA (also an MAO inhibitor) resulted in an initial 'burst effect', but levels well above control values are maintained for a relatively long period of time. The double prodrug feature of DPGPEA might contribute to these properties. Both PGPEA and DPGPEA are unique in that they represent novel MAO inhibitors that cause marked elevations of PEA levels without markedly increasing concentrations of other amines.

Availabilities (AUCs) of the prodrugs of PEA in brain, blood and liver were summarized in Fig. 45. In brain, CPPEA had a greater AUC than the others, while in liver CPPEA displayed a similar AUC to PGPEA and DPGPEA. Similar AUC values for CPPEA were observed in brain and liver, whereas the AUC values of CEPEA, PGPEA and DPGPEA in liver were higher than those in brain. However, the concentrations of CEPEA in liver were much less than those of PGPEA and DPGPEA. Despite this peripheral tissue localization, CEPEA, PGPEA and DPGEA provided higher PEA levels in brain than did CPPEA.

The present investigation has clearly demonstrated that it is possible to selectivity (i.e. without increasing other amines) increase levels of PEA in the CNS without the use of the usual MAO inhibitors by employing N-alkylated analogs of PEA as prodrugs. Levels of PEA in brain after these prodrugs were similar to or markedly higher than those obtained after administration of MAO inhibitors. Although PGPEA and DPGEA were shown to be MAO inhibitors, they did cause marked increases in PEA levels which were larger than those expected from MAO inhibitors. These prodrugs can thus be employed as novel pharmacological tools with which to study the functional role of PEA in the CNS.

In the present investigation, levels of PEA and its prodrugs have been measured in brain, blood and liver to help in characterization of prodrug distribution. The detection of a drug and its metabolite(s) in the CNS does not necessarily mean formation of the metabolite occurs in the CNS. The metabolite could be formed in liver and be transported to brain unless its physicochemical properties prevent or preclude such an event. But this observation should not lead one to misconstrue that brain lacks metabolic capacity for exogeneous compounds (xenobiotics). Several studies have demonstrated the metabolic potential of brain, and, to quote Gorrod (1978), "brain has conjugating systems present as well as those able to carry out Phase I metabolic pathways" (Phase I reactions include dealkylation, deamination, oxidation, reduction, and hydrolysis).

The levels of PEA and its prodrugs have been measured employing GC-ECD following extractive derivatization with PFBC, and in preliminary studies PFBSC was demonstrated to derivatize these prodrugs under aqueous conditions. The concept of using perfluoroacylating agents for extractive derivatization is a relatively recent one (see Table 7). This procedure combines extraction and derivatization in a single step, thereby reducing total analysis time. A modification of the method of Nazarali _et al._ (1986a) has been employed for this purpose. Levels of PEA in untreated rat tissues were comparable to those reported earlier employing TLC-HRMS techniques (Philips, 1984). The GC-ECD techniques employed in present investigations offer simple, sensitive and reproducible assay procedures as a viable alternative to GC-MS techniques which require costly equipment as well as highly trained personnel.

Effects of administration of equimolar doses of PEA and its prodrugs (0.1 mmol/kg, i.p.) on putative neurotransmitter amines were examined by measurement of brain levels of NA, DA, 5-HT, DOPAC, HVA and 5-HIAA at different times after administration. In comparison to transient increases in brain PEA levels produced by administration of PEA itself, administration of prodrugs caused sustained elevations in PEA levels. Differences in patterns of changes in neurotransmitters and their metabolites after PEA and its prodrugs suggest possible neurochemical effects of prodrugs such as abilities to affect uptake and release of neurotransmitters and to inhibit MAO (for PGPEA and DPGPEA). These neurochemical effects of prodrugs cannot be unequivocally delineated from those due to altered PEA levels in _in vivo_ situations. The results of _in vitro_ uptake and release experiments with the prodrugs in combination with _in vivo_ experimental results as described here may help in clarifying this situation.

F. Tissue Levels of T and CET

Because of the encouraging results with the prodrugs of PEA, it was decided to perform a preliminary study with a potential prodrug of T, another bioactive amine which has been proposed to be involved in the etiology of depression and is known to be increase markedly in brain following administration of MAO inhibitors (see Introduction section of this thesis). The N-cyanoethyl analog of T was chosen for this preliminary investigation.

The levels of T and CET after administration of T or CET were determined by aqueous pentafluorobenzenesulfonylation followed by GC-ECD. Using this technique, levels of T in control tissues could not be accurately quantitated, but they were less than 1 ng/g in each case and were assumed to be 0.5, 0.2 and 0.7 ng/g for brain, blood and liver respectively, as found by Philips (1984) using high resolution MS. After i.p. administration of T, levels of T in brain, blood and liver increased significantly above control levels, but the increases were not uniform. The fact that peak levels in brain were nearly 4 times and 45 times lower than those in blood and liver respectively suggests reduced penetration of T across the blood-brain barrier relative to PEA and/or existence of a first-pass metabolism in the periphery. Because of its lipophilicity, T would be expected to enter brain rapidly, and Oldendorf (1971) reported that T crosses the blood-brain barrier relatively easily, although in this case T was administered through the intracarotid artery. Peak levels at 5 min in brain suggest rapid entry, but the low levels relative to those seen with PEA would suggest that only a small fraction of the administered dose gains access to brain. Similar conclusions were reached in a recent study (Dyck and Boulton, 1986) employing a highly sensitive and specific TLC-HRMS technique. Indirect evidence for limited CNS absorption of intraperitoneally administered T comes from earlier behavioral studies, in which T elicited CNS stimulant effects at relatively high doses approaching toxic doses or only upon pretreatment with MAO inhibitors (Green and Sawyer, 1960; Vogel and Cohen, 1977; Luscombe et al., 1982). The patterns of decreases in T levels in brain, blood and liver were similar to those observed by Dyck and Boulton (1980) and Wu and Boulton (1973) in that there was a rapid elimination phase followed by a slower elimination phase. Wu and Boulton (1973) reported the half-life elimination (β) value for [^{3}H]-T in brain to be about 34 min (assuming linearity in T concentrations from 10-60 min). In the present investigation employing the method of residuals (Section II.H), two half-lives were observed ($t1/2\alpha$=12.5 min, $t1/2\beta$=90 min, Table 17).

Analysis of blood T levels (5-120 min) after administration of T or CET (0.1 mmol/kg, i.p.) indicated a significant difference between effects of drug treatments [F(1,55)=6.85, P<0.0025], a significant effect of time and a significant interaction between treatment and time [F(5,55)=8.31, P<0.001 and F(5,55)=6.65, P<0.001 respectively]. Similar analysis in brain indicated a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [F(1,60)=7.25, P<0.0025; F(5,60)=44.76, P<0.001 and F(5,60)=16,84, P<0.001 respectively]. Equivalent analysis in liver indicated a significant difference between effects of drug treatment, a significant effect of time and a significant interaction between treatment and time [F(1,64)=25.13, P<0.001; F(6,64)=47.24, P<0.001 and F(6,64)=38.49, P<0.001 respectively]. After administration of CET, T levels increased slowly and peak levels in brain, blood and liver were significantly lower than those observed after an equimolar dose of T, indicating that prodrug administration caused delayed release of T in these tissues. The levels of T also decreased at a slower rate after CET administration than after T administration. Levels of T after T treatment decreased rapidly during the α-phase and this phase accounted for nearly 80%-90% of available T, whereas after CET treatment T levels in brain and liver (but not blood) decreased monoexponentially (Table 17). There was no change in overall T availability in brain after CET and T treatments, whereas in blood and liver, CET treatment resulted in decreased overall availability. Two reasons can be advanced to explain this observation:

1. Metabolic N-dealkylation of CET may not occur at a rate sufficient enough to sustain T levels. Tryptamine has been shown to have a rapid turnover rate (Durden and Philips, 1980) and it is a good substrate for MAO. If N-dealkylation of CET to T proceeds at a rate slower than deamination of T by MAO, CET treatment would result in relatively small increases in T availability.
2. The second possibility is that CET might be eliminated rapidly so that CET levels are insufficient to elicit a prodrug effect. Half-life calculations (Table 17) indicated that CET was eliminated faster than T from brain, blood and liver and this rapid elimination of CET might be responsible for the relatively weak prodrug effect.

In *in vivo* experiments, the extent of dealkylation is difficult to determine, but assuming the ratio of the AUCs of T to CET as an index of the extent of N-dealkylation, about 0.5% of available CET was converted to T in brain, while in liver the ratio was 3%. This indicates limited *in vivo* conversion of CET to T compared to the conversion of the prodrugs of PEA to PEA. Thus limited N-dealkylation and rapid elimination of CET might contribute to the relatively small increases in brain T levels seen after CET treatment. But these increases were sustained, and detectable levels of T could be found in brain, blood and liver even at 180 min after injection, whereas after T injection, levels of T were detectable only at 120 min. *In vitro* studies indicated fairly potent inhibition of MAO-A by CET relative to inhibition of MAO-B (Section III.G), but *in vivo* MAO inhibition data at 60 min after i.p. injection of CET showed that MAO activity was nearly normal, suggesting that inhibition by CET is rapidly reversible.

G. Levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA After Administration of T and CET

Interactions of T and CET with levels of neurotransmitter amines and their metabolites were determined employing HPLC-EC. Administration of T influenced DA, HVA, DOPAC, 5-HT levels without affecting NA and 5-HIAA levels, while injection of CET altered DA, DOPAC, 5-HT and 5-HIAA levels without affecting NA and HVA levels (see Figs. 72-77).

Analysis of NA levels (5-180 min) after administration of T or CET (each 0.1 mmol/kg, i.p.) indicated no significant difference between effects of treatments, no significant effect of time and no significant interaction between treatment and time [F(1,86)=0.001, P>0.1; F(7,86)= 1.89, P>0.1 and F(7,86)=0.93, P>0.1 respectively]. Data in Fig. 72 clearly indicate that T and CET have limited effects on the NA system.

Statistical analysis of DA levels after administration of T or CET indicated a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [$F(1,86)=87.43$, $P<0.001$; $F(7,86)=9.09$, $P<0.001$ and $F(7,86)=4.43$, $P<0.001$ respectively]. Similar analysis of brain HVA levels revealed a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [$F(1,86)=61.71$, $P<0.001$, $F(7,86)=11.56$, $P<0.001$ and $F(7,86)=7.15$, $P<0.001$ respectively]. Equivalent analysis of DOPAC levels indicated a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [$F(1,83)=306.62$, $P<0.001$; $F(7,83)=8.35$, $P<0.001$ and $F(7,83)=17.03$ respectively]. Calculations of DA turnover [ratio of sum of DOPAC and HVA (% control) to DA (% control), baseline value of 2] indicated that T administration resulted in increased turnover values (in the range of 2.5-3.3) from 5-90 min; the turnover value decreased to the baseline value by 180 min. In contrast, CET administration resulted in decreases in DA turnover, with values ranging from 1.06 to 1.5 between 5 to 120 min, and the turnover increased to the baseline value by 180 min. This would indicate opposite effects of T and CET on the dopaminergic system (see Figs. 73-75).

Analysis of 5-HT levels after T and CET treatments indicated a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [$F(1,84)=59.06$, $P<0.001$, $F(7,84)=3.08$, $P<0.005$ and $F(7,84)=3.15$, $P<0.005$ respectively]. Equivalent analyses of 5-HIAA levels also indicated a significant difference between effects of treatments, a significant effect of time and a significant interaction between treatment and time [$F(1,87)=84.87$, $P<0.001$, $F(7,87)=3.82$, $P<0.005$ and $F(7,87)=7.04$, $P<0.001$ respectively]. Administration of T slightly increased 5-HT turnover [defined by the ratio of 5-HIAA to 5-HT (% controls), baseline value of 1.0] in the range of 1.1 to 1.34 from 5-180 min, whereas CET administration decreased 5-HT turnover (values in the range of 0.5-0.9) up to 120 min, after which turnover increased to the baseline value. These opposing effects are demonstrated in Figs. 76 and 77.

Administration of T to various experimental animal species produces behavioral effects (reviewed in Jones, 1982) that appear to be mediated through serotoninergic mechanisms or through a direct effect on receptors for T as these actions were blocked by methysergide (Dewhurst and Marley, 1965). The present study indicates that T may also have strong effects on DA systems as well. Injection of T depleted DA levels and even at 180 min after T injection, DA levels were below control levels.

Baker and Yasensky (1981) observed DA release from striatal prisms by T; this was a weaker release than that seen after p-TA and PEA. Squires et al. (1978) observed that DA antagonists can block behavioral effects of T, indicating dopaminergic involvement in addition to a serotoninergic role. Marsden (1980) suggested that although initial behavioral effects seen after α-methyltryptamine were serotoninergic in nature, later effects appear to be mediated by both DA and 5-HT systems. Decreases in DA levels accompanied by increases in DOPAC and HVA levels in the present study strongly suggest an increase in DA turnover at initial times. This may be due to the releasing effects mentioned above or due to inhibiting effects of T on DA uptake (Heikkila et al., 1974; Baker and Yasensky, 1981). Increases in DOPAC suggest that DA release by T predominates over uptake inhibition. In contrast, DA levels after CET administration were not different from control levels. These were accompanied by marked decreases in DOPAC levels without changes in HVA; these changes taken together indicate a decrease in DA turnover. Decreases in DOPAC levels without changes in DA suggest possible uptake inhibition, but uptake blockade with enhanced release should increase HVA levels. This might be due to predominance of uptake blockade over release although the interactions of CET with the DA system should also be considered in addition to changes due to T (formed from CET) itself.

Levels of 5-HT after T treatment decreased below controls only at 60 min, while 5-HIAA levels remained unchanged. Treatment with CET increased 5-HT levels only at 5 min, while 5-HIAA levels decreased initially and increased at later times. Horn (1973) and Baker *et al.* (1977, 1980c) reported inhibition of 5-HT uptake by T in rat brain tissue preparations, and Born *et al.* (1972) and Airaksinen *et al.* (1980) reported 5-HT uptake inhibition by T in human platelets. Baker *et al.* (1977, 1980c) and Ennis and Cox (1982) also observed a potent releasing action of T on 5-HT from rat brain tissue preparations. Weak *in vivo* inhibition of MAO after CET despite relatively potent MAO-A inhibition *in vitro* suggest the possibility of reversible *in vivo* inhibition of MAO-A after CET. This might account for a small initial increase of 5-HT at 5 min after CET treatment and a decrease in 5-HIAA levels up to 30 min. Changes in T levels along with CET might also affect uptake and release of 5-HT. In contrast to marked changes in the DA system following T treatment, changes in 5-HT levels were minimal. Treatment with CET appears to influence both DA and 5-HT systems, to varying degrees. Further studies are required to delineate the effects of prodrug from its metabolite with regard to interactions with neurotransmitter amines.

In summary, T administration resulted in differential increases in tissue T levels, and the pharmacokinetic data suggest reduced ability of T relative to PEA to cross the blood-brain barrier following i.p. administration and/or existence of a first-pass metabolism in the periphery. Administration of CET elevated and sustained T levels in tissues, in contrast to the rapid decreases in T levels seen after T injection itself. The increases in T levels after CET were not as high as the increases in PEA levels after CEPEA treatment. The pharmacokinetics of CET might be responsible for such an observation. Neurochemical studies suggested fairly potent effects on DA and 5-HT systems relative to effects on NA systems after T and CET injections, indicating that the prodrug itself may be neurochemically active.

H. In Vitro Inhibition of MAO by Structural Analogs of Tranylcypromine (TCP)

During the course of the present investigation, inhibition of MAO *in vitro* by structural analogs of TCP (see objectives and hypotheses, Section I.L 94-97) has been examined using MAO-A- and -B-specific substrates (Rao *et al.*, 1986b). These studies revealed that some structural analogs of TCP were equipotent with or more potent than TCP itself (Table 18). Earlier, Zirkle *et al.* (1962), using potentiation of T-induced convulsions as an index of MAO inhibition potency *in vivo*, reported that electron-withdrawing and electron-donating substituents did not increase MAO inhibitory activity in TCP analogs. During the course of the present investigation, FTCP and naphthylcyclopropylamine (NCP) were found to be more potent than TCP as MAO inhibitors. Several phenyl ring-substitued analogs of TCP, namely 3,4-methlenedioxy-

TCP (MDTCP), 4-methoxy-TCP (MTCP), and 3-trifluoromethyl-TCP (TTCP), were equipotent with TCP at inhibiting MAO-A and MAO-B in vitro. The techniques employed by Zirkle et al. (1962) did not yield information on the inhibitory potential against MAO-A and -B. Since the discovery of multiple forms of MAO, no further studies had been conducted to elucidate the inhibitory activity of the TCP analogs. The present study confirmed earlier reports indicating higher potency of (+)-TCP than (-)-TCP at inhibiting MAO activity (Zirkle et al., 1962; Fuentes et al., 1976; Reynolds et al., 1980; Hampson et al., 1986); similar potencies had also been observed in in vivo studies (Fuentes et al., 1976; Reynolds et al., 1980; Hampson et al., 1986). N-Acylation or N-alkylation in general resulted in decreased in vitro activity against MAO-A and -B, although the extent varied. N-Acetyl-TCP (ATCP) was about 1000 to 10000 times weaker than TCP as an inhibitor of the two forms of MAO in vitro, and similar potencies were observed for carbamate analogs of TCP [ethoxycarbonyl-TCP (ECTCP), isobutoxycarbonyl-TCP (ICTCP), see Fig. 12)]. Calverley et al. (1981) reported the presence of ATCP in rat brain following TCP administration, but its MAO inhibitory activity was not determined. Kang and Young (1984a) identified ATCP in rat urine and Kang and Choi (1986) characterized its MAO inhibitory potential in vivo. These latter authors found that administration of a 0.4 mmol/kg (i.p.) dose in rats resulted in a gradual increase in MAO inhibition (determined by 5-HT deamination) from minimal inhibition at 0.5 hr to about 90% inhibition between 6-12 hr; 74% inhibition was still observed by 24 hr. A nearly 1000-fold weaker in vitro potency of ATCP in comparison to TCP (Rao et al., 1986b; Kang and Choi, 1986) and the gradual increase in MAO inhibition in vivo suggests biotransformation to TCP and/or some other active metabolite *in vivo*. Kang and Choi suggested that polymorpic N-acetylation of TCP might be responsible for differential side effect profiles as well as therapeutic benefits in the patient population. If *in vivo* N-deacetylation of ATCP to TCP is a major metabolic process, then ATCP could well be conceived as a prodrug of TCP.

Carbamate analogs of phenylethylamines have been shown to undergo *in vivo* N-dealkylation to generate parent amines (Verbiscar and Abood, 1970; Baker *et al.*, 1984b), and Nazarali (1984) demonstrated that ECTCP was metabolized to TCP, thus acting as a prodrug. Zirkle *et al.* (1962) observed that the N-benzylcarbamate derivative of TCP was nearly twice as potent an MAO inhibitor as TCP *in vivo* and recently Kang and Young (1984) demonstrated the biotransformation of this carbamate to TCP *in vivo*. It is likely that ICTCP (Table 18) would also be metabolized to TCP *in vivo*. Among the N-alkyl analogs of TCP, CETCP, the N-cyanoethyl analog of TCP was a potent MAO inhibitor in its own right *in vitro* and it was demonstrated to be a good prodrug *in vivo* (Baker *et al.*, 1984d; Nazarali *et al.*, 1986c). N-Propargyl-TCP was found to be a weaker MAO inhibitor than TCP *in vitro*. Pretreatment with MAO inhibitors attenuates the increase in brain levels of GABA seen following the administration of PLZ and Popov *et al.* (1967) observed that *in vivo* inhibitory potency of MAO inhibitors paralleled their abilites to attenuate this increase. Using such a pharmacological method, Popov *et al.* (1967) observed that PGTCP was less potent than TCP, and the present investigation confirms this earlier observation. Encouraging results with N-propargyl analogs of PEA led to the detailed examination of PGTCP as a possible prodrug of TCP *in vivo* (see Section IV.J). Compounds with the N-propargyl moiety are known to inactivate MAO through irreversible inactivation as well as to undergo *in vivo* N-dealkylation to generate parent amines (see Section IV.A.4). It was hoped that PGTCP would be a novel MAO inhibitor and a prodrug of a known MAO inhibitor.

I. Comparative Pharmacokinetic and Neurochemical Studies on TCP and FTCP

In vitro tests indicated that FTCP was nearly 10 times more potent than TCP at inhibiting MAO and this prompted a comparative neurochemical and pharmacokinetic study on TCP and FTCP. A novel GC-ECD analytical technique for the analysis of TCP in rat tissues (blood, brain, liver, heart, kidney, lung, heart and spleen) was developed (Rao *et al.*, 1986a) and this method has been employed for the analysis of FTCP in these tissues (Coutts *et al.*, 1986a). The method employed PFBSC (see objectives and hypotheses, Section I.L) as a novel reagent to extract and derivatize TCP or FTCP under aqueous conditions. In preliminary studies, this method was also shown to be applicable to the derivatization of TCP analogs such as MTCP, MDTCP, TTCP, NCP, CETCP and PGTCP (see Fig. 12).

Several methods have been developed to analyse TCP in biological samples. Turner *et al.* (1961) employed a fluorometric method to determine TCP elimination in human urine samples. A radioenzymatic assay method was developed by Fuentes *et al.* (1975) to quantitate TCP isomers in rat brain following the administration of the individual isomers. Baselt *et al.* (1977a) employed GC with flame ionization detection to analyze TCP in underivatized form in autopsy specimens. The same group (Baselt *et al.*, 1977b) also utilized anhydrous trichloroacetylation followed by GC-ECD to measure TCP levels in human plasma samples. Lang et al. (1978) utilized dansyl chloride to derivatize enantiomers of TCP; this was followed by TLC separation and fluorometric measurement to quantitate TCP isomer levels in human plasma and urine. A GC-NPD technique was used by Bailey and Baron (1980) for the analysis of TCP levels in human plasma and urine samples; this followed extraction into an organic solvent under alkaline pH and anhydrous heptafluorobutyrylation. Aqueous acetylation followed by anhydrous pentafluorpriopionylation or pentafluorobenzoylation and analysis by GC-ECD have also been used for quantitation of TCP in brain and/or urine samples (Calverley et al., 1981; Hampson et al., 1984). Baker et al. (1985) employed aqueous trichloroacetylation followed by GC-ECD analysis to quantitate TCP levels in rat brains. Analysis by GC-ECD has also been used for measurement of TCP in human plasma after derivatization with PFBC under anhydrous conditions (Youdim et al.; 1979; Reynolds et al., 1980). In a recent report, Edwards et al. (1985) employed anhydrous pentafluoropropionylation followed by a GC-CI-MS-SIM technique for analysis of TCP levels in human plasma.

The aqueous pentafluorobenzensulfonylation procedure developed in the present investigation combines extraction and derivatization into a single step and offers a rapid, sensitive technique for the analysis of TCP in biological samples. This method can also be employed for the analysis of TCP in urine samples (rat and human), is applicable to analysis of PEA in brain and urine samples (Baker et al., 1986a) and has the added advantage of permitting simultaneous analysis of TCP, PGTCP and PEA in the same sample.

As indicated in the Introduction (see Section I.L), one of the objectives of the project was to study the pharmacokinetics and neurochemistry of FTCP after intraperitoneal injection and to compare the results to those obtained with TCP after injection of an equimolar dose. It was hypothesized that FTCP, because of the presence of a fluorine atom in the *para* position of the phenyl ring would be protected from ring hydroxylation and thus would attain higher brain levels than TCP and be cleared from the brain more slowly than TCP.

After intraperitoneal administration TCP or FTCP, peak levels of each drug were observed in all three tissues studied at 30 min after administration (Figs. 78-80). Higher peak levels in brain than in blood at this time suggest fairly rapid entry of TCP and FTCP into brain. Significantly greater AUCs of TCP and FTCP in brain and liver than those in blood suggest considerable diffusion into tissue compartments from blood. Similar kinetic profiles in brain and liver for TCP and FTCP indicate a lack of preferential tissue localization. Relatively high concentrations of FTCP and TCP were observed in a variety of tissues at 30 min. The significantly higher AUC of FTCP compared to TCP in brain and liver suggests slower elimination of FTCP in comparison to TCP; and this is confirmed by half-life calculations (Table 19).

Comparison of TCP and FTCP levels in blood by two-way analysis of variance indicated no significant difference between the effects of the two drug treatments [F(1,54)=0.62; P>0.1] and a significant effect of time [F(6,54)=21.06, P<0.001), but no significant interaction between drug treatment and time (significant interactions between drug treatments and time are indicative of differences in concentration-time profile between drugs) [F(6,54)=0.74; P>0.1]. In brain, similar analyses revealed a significant difference between the effect of drug treatment

[$F(1,63)=34.86$; $P<0.001$], a significant effect of time [$F(6,63)=97.92$; $P<0.001$] and a significant interaction between drug treatment and time [$F(6,63)=7.31$; $P<0.001$]. A comparison of TCP and FTCP levels in liver indicated a significant difference between the effects of drug treatment [$F(1,63)=21.80$; $P<0.001$], a significant effect of time [$F(6,63)=100.08$; $P<0.001$], and a significant interaction between drug treatment and time [$F(6,63)=3.02$; $P<0.05$]. These differences are reflected in the kinetic profiles illustrated in Figs. 78-80 and Table 19. FTCP attained higher peak levels than TCP and was eliminated at a slower rate than TCP from brain and liver but not from blood.

Pharmacokinetic analysis of TCP and FTCP levels indicated biphasic elimination in brain, blood and liver with a fast decline phase [with a half-life (t1/2) of 0.9-1.4 hr for TCP and 0.6-0.8 hr for FTCP] and a slower decline phase (t1/2 of 3-11.0 hr TCP and 7-11.5 hr for FTCP). These data are somewhat at variance with reported t1/2 values (β) of TCP. Youdim *et al.* (1979) reported an elimination t1/2 (β) of 3.5 hr in plasma from a patient overdosed with TCP, and Baselt *et al.* (1976) observed a serum t1/2 (β) of 1.8-2.0 hr (based on serum concentrations 1-5 hr post administration). Turner *et al.* (1967) found a β value of 2.7 hr in humans, while Weber *et al.* (1984, calculated from data presented therein) observed a β value of about 2.0-2.1 hr in humans (calculated from levels for 1-8 hr post administration). A β-value of 2.4 hr (from plasma levels 1-10 hr post administration) was reported by Edwards *et al.* (1985). In a study in rat brain, Hampson *et al.* (1986) observed a β-value of about 0.76 hr for (-)-TCP and 1.45 hr for (+)-TCP (from brain levels at 0.5-6 hr postinjection). Nazarali (1984) observed a β-value of 1.67 hr in rat brain (based on brain levels from 2-4 hr).

In the reports cited above TCP levels were not monitored for longer times (up to 24 hr) as was done in present investigation; the slower elimination (β) phase in fact was observed after 12 hr. By neglecting this slower elimination phase (assuming linearity in that range), calculations of t1/2 from levels of TCP (FTCP) in tissues between 0.5-12 hr gave t1/2 values of TCP and FTCP in all three tissues between 2.0 and 3.5 hr; these values agree with the reports cited above. However, pharmacokinetic analysis reveals two distinct phases of elimination; although nearly 90%-95% of available TCP (FTCP) after administration was eliminated during the α-phase, the slower elimination phase should not be ignored altogether.

Recent metabolic studies on TCP indicated that it undergoes aromatic hydroxylation at the para position (Baker et al., 1986b), N-acetylation (Calverley et al., 1981) and N-acetylation and hydroxylation combined (Kang and Young, 1984a; Kang and Choi, 1986). Baker et al. (1986b) reported that pretreatment with known blockers of aromatic hydroxylation such as iprindole, chlorpromazine, trifluoroperazine and SKF-525A caused a marked increase in TCP brain levels (2-4 times). The presence of a fluorine atom at the para position of the aromatic ring should block the metabolic hydroxylation, and this was the reason for preparing FTCP. It was hoped that FTCP, which was shown earlier in this investigation to be an extremely potent MAO inhibitor (see Section III.I of this thesis), would be eliminated slower than TCP from brain. From the AUC measurements, it is evident that FTCP provides significantly greater availability of TCP in brain and liver at the dose and time intervals used in this study. This marked increase in availability could be due to blockade of metabolic hydroxylation. Higher concentrations of FTCP than of TCP in tissues after administration of equimolar doses, slower elimination of FTCP and the report by Baker et al. (1986b) indicating a 2-4 fold increase in TCP levels following pretreatment with drugs such as iprindole, chlorpromazine, trifluoroperazine and SKF-525A clearly indicate that ring hydroxylation could be an important metabolic pathway for TCP in the rat.

The in vivo inhibition of MAO in brain and liver indicates that nearly 85% inhibition of MAO-A and -B was attained within 0.5 hr of drug administration and the pattern of inhibition remained more or less unchanged over a 24 hr period (Tables 20 and 21), suggesting irreversibility of enzyme inactivation. At the dose (0.1 mmol/kg, i.p.) employed there were no quantitative differences in MAO inhibition in vivo by TCP and FTCP.

As shown in Table 18, FTCP was approximately 10 times more potent than TCP at inhibiting both MAO-A and MAO-B in vitro. Although in in vitro studies 2-(4-fluorophenoxy)cyclopropylamine, a compound closely related structurally to FTCP, was found to be equipotent to TCP with regard to inhibition of MAO using TA as a substrate (Fenkelstein et al., 1965), FTCP had not been tested previously for its MAO-inhibiting properties. Tranylcypromine is a nonspecific suicidal inactivator of MAO (Silverman, 1983). A decrease in the pKa value by fluorine substitution in the aliphatic side-chain has been reported to render some fluoro analogs of PEA and amphetamine better substrates for and better inhibitors of MAO, respectively, than their nonfluorinated analogs (Fuller and Molloy, 1976). Since FTCP and TCP have similar pKa values, changes in

FTCP over TCP with regard to inhibition of MAO-A and -B in vitro. Perhaps altered kinetics of enzyme-substrate interactions may be responsible.

Preliminary studies were undertaken to determine the effects of administration of TCP and FTCP on the levels of two trace amines, PEA and T, and on the levels of the neurotransmitter amines NA, DA and 5-HT. The levels of PEA and T in biological samples were quantified by modifications to methods reported by Baker et al. (1986a) and Rao et al. (1986a). This method employed aqueous pentafluorobenzenesulfonylation followed by GC-ECD to quantitate these amines, and allowed quantitation of PEA levels in control tissues in a simple assay procedure. The results obtained with this techique were in close agreement with those obtained from GC-MS or TLC-HRMS techniques (see Table 22). The procedure has also now been applied to the analysis of free PEA in urine samples of control subjects. Urinary excretion values (24 hr) in 12 healthy volunteers (6 males and 6 females) were 3.4 ± 0.35 µg/g of creatinine (mean ± S.E.M.), and these values are in close agreement with those reported using a TLC-HRMS method (Slingsby and Boulton, 1986). Thus the method has some advantages over another procedure (Martin and Baker, 1977) used in our laboratories. The Martin and Baker method, which utilizes aqueous acetylation followed by pentafluoropropionylation under anhydrous conditions, works very well for analysis of PEA in brain, but does not provide for simultaneous analysis of the N-alkylated prodrugs of PEA and gives urinary values of PEA that are somewhat higher (Coutts et al., 1981) than those found by mass spectrometric procedures.

As mentioned in the Introduction section, there is considerable controversy in the literature about control values of T in brain. However, the reported mass spectrometric methods (Philips et al., 1979; Warsh et al., 1977; Artigas and Gelpi, 1979; Gelpi and Artigas, 1984), which should be the most specific and sensitive, give values which are in good agreement with one another, at about 0.5 ng/g. A previous GC-ECD procedure used in our laboratories utilized aqueous acetylation followed by reaction with pentafluoropropionic anhydride for analysis of T (Baker et al., 1979, 1981). This procedure had the advantage of providing for simultaneous quantitation of 5-HT in brain and urine (Baker et al., 1980) and of PEA in brain (Baker et al., 1981); however, its limit of sensitivity was 1 ng/g, slightly too high to permit accurate quantitation of T in control brain and liver, although it permitted quantitation in these tissues from MAO inhibitor-treated rats and in control urine from rats and humans. In addition, this procedure did not provide for simultaneous analysis of levels of CET. The pentafluorobenzoylation method, utilized by Wong (1985) to analyze T in food products, was not satisfactory in brain samples because of the presence of interfering peaks. The aqueous pentafluorobenzenesulfonylation technique developed in the present project permitted simultaneous analysis of T and CET, was also applicable to analysis of PEA (Baker et al., 1986), TCP (Rao et al., 1986a) and FTCP (Coutts et al., 1986a), and had the same limit of sensitivity (1 ng/g) as the acetylation/pentafluoropropionylation method. Thus it was possible to show that control brain and liver levels of T were <1 ng/g and, for calculation purposes, and it was assumed these levels were 0.5 and 0.7 ng/g respectively, as indicated by high resolution mass spectrometric procedures.

There were marked increases in the levels of PEA in brain, blood and liver after TCP and FTCP treatment. Statistical analysis of brain PEA levels following TCP and FTCP administration indicated a significant difference between the effects of the two drug treatments [F(1,73)= 14.31, P<0.001] and a significant effect of time [F(7,73)=7.27, P<0.001], but no significant interaction between drug treatments and time [F(7,73)=0.97, P>0.1] was observed. The fluorinated analog FTCP caused greater elevations in brain PEA levels than did TCP at 0.5, 1.5, 16 and 24 hr (Fig. 82).

Statistical analysis of blood PEA levels after the injection of TCP and FTCP revealed no significant overall difference between the effects of the two drug treatments on PEA levels [F(1,64)=1.58, P>0.2], but a significant effect of time [F(7,64)=22.65, P<0.001] and a significant interaction of time and drug treatments [F(7,64)=24.35, P<0.001] were noted. The changes in blood PEA levels were not as pronounced as they were in brain and liver. Administration of TCP caused transient increases in blood PEA levels whereas FTCP treatment resulted in gradual changes in PEA levels, indicating differences of the effects on PEA of the two drug treatments (Fig. 83).

Transient changes in blood PEA levels after TCP may have some functional significance since PEA is a sympathomimetic amine and is known to release NA from its stores in the periphery. This might contribute to the side effect profile of TCP, whereas gradual increases in PEA levels after FTCP might reduce such problems; it would be interesting to study the effects of these analogs in other peripheral organs in addition to the liver (e.g. heart).

Increases in liver PEA levels were greater than those in brain and blood. Statistical analysis of liver PEA levels following the administration of TCP and FTCP indicated a significant difference between effects of the two drug treatments [$F(1,69)=15.04$, $P<0.001$], and a significant effect of time [$F(7,69)=6.09$, $P<0.001$], but no significant interaction between drug treatment and time [$F(7,69)=1.27$, $P>0.05$]. Administration of FTCP increased PEA levels above those seen after TCP (Fig. 84).

Levels of T, another trace amine, were also measured in brain and liver. Because of the presence of an as yet unidentified interfering peak in blood following administration of the drugs, a detailed analysis of blood T levels could not be carried out. Statistical analysis of brain T levels following administration of TCP and FTCP indicated a significant difference between the effects of the two drug treatments [$F(1,46)=4.87$, $P<0.005$] and a significant effect of time [$F(4,46)=3.98$, $P<0.05$], but no significant interaction between drug treatments and time [$F(4,46)=2.09$, $P>0.05$]. The relative increases in T levels over control levels in brain after TCP and FTCP administration were greater than those of PEA. The overall increases in T levels in brain after TCP administration were greater than those after FTCP (Fig. 85). Peak levels of T after TCP were seen at earlier times than after FTCP, although the peak levels attained in both cases were comparable. By 16 hr T could be quantitated in only one rat brain in a group of 5-6 in the TCP-treated group, whereas after FTCP treatment T could be measured even at 24 hr.

Statistical analysis of T levels in liver following the administration of TCP and FTCP indicated no significant difference in effects of the drug treatments [$F(1,60)=3.58$, $P>0.05$], a significant effect of time [$F(6,60)=8.08$, $P<0.001$], and a significant interaction between drug treatments and time [F(6,60)=2.78, $P<0.025$]. Administration of TCP and FTCP caused greater T increases in liver than in brain (Fig. 86). Peak levels after TCP were higher than those after FTCP ($P>0.05$), but by 24 hr, T levels were higher in the FTCP-treated group than in the TCP-treated animals.

The levels of PEA and T were markedly increased after TCP and FTCP treatment. 2-Phenylethylamine is an excellent substrate for MAO-B, whereas T appears to be metabolized predominantly by MAO-A [although there is some controversy in this regard (see Section I.J.2)]. These trace amines have been proposed to function as neuromodulators and their functional deficiencies have been implicated in the etiology of affective disorders (Dewhurst, 1968, 1984; Boulton, 1976a, 1976b,, 1984, see Section I.J). Earlier studies in which levels of these trace amines have been measured after MAO inhibitor treatment (Dewhurst, 1968; Boulton et al., 1973; Axelrod and Saavedra, 1974; Philips and Boulton, 1979; Philips et al., 1980; Baker et al., 1984a; Philips, 1984) indicated marked increases in the levels of these trace amines in comparison to increases in NA, DA and 5-HT, and these increases have been proposed to contribute in part to the therapeutic efficacy of this class of drugs (Dewhurst, 1968; Boulton, 1973). There were differences in the relative increases in the levels of PEA and T after TCP and FTCP treatment. While TCP was more potent at increasing overall T levels, FTCP was more potent at increasing overall PEA levels (see above discussion). Both PEA and T are lipophilic compounds and the levels of these amines depends upon the availability of precursors in brain as well as an MAO inhibition in brain and periphery. Lung contains an active uptake mechanism for PEA by MAO-B (Ben-Harare and Bakhle, 1980) and liver contains quantitatively greater MAO activity than brain (Gorkin, 1983); these mechanisms might reduce transport of PEA from peripheral tissues to brain. Recent evidence (Dyck and Boulton, 1986; present investigation, Section III.F.1) indicated that intraperitoneally administered T did not cross the blood-brain barrier as effectively as PEA, which may be due to the existence of first-pass metabolism in the periphery. Administration of MAO inhibitors might result in inhibition of these "metabolic-sink" mechanisms, thereby greatly improving availability of amines to brain from the periphery in addition to decreasing their degradation in brain. Grahame-Smith (1971) and Tabakoff _et_ _al._ (1977) have reported increases in levels of tryptophan, the precursor for T, in rodent brain after TCP administration. The mechanism of the increase is not clear, but it might be due to facilitation of tryptophan transport into brain by TCP, since other MAO inhibitors have been reported to influence transport of amino acids (Rafaelson, 1976). Interestingly, in studies with individual TCP enantiomers, Smith and Peterson (1982) did not find an increase in tryptophan levels in rat brain. Chronic administration of TCP has been reported to induce AADC activity (Robinson _et al._, 1979), which facilitates conversion of tryptophan to T. Although acute studies were not conducted to determine this enzyme's activity, facilitated transport of precursor amino acid along with diffusion of T formed in the periphery into the brain might contribute to the observed levels in brain. Differences in T levels after TCP and FTCP treatments may therefore be due in part to differences in the abilities of the two drugs to affect amino acid transport into brain and their possible influences on brain AADC activity. Further studies are required to elucidate the underlying mechanisms.

In the present study, HPLC-EC was employed to measure the levels of NA, DA and 5-HT in brain and determine the effects of TCP and FTCP on those levels. Statistical analysis of NA levels after TCP and FTCP administration revealed no significant difference between effects of the drug treatments [F(1,78)=3.15, P>0.05], a significant effect of time [F(7,78)=58.76, P<0.001], and a significant interaction between time and drug treatments [F(7,78)=10.06, P<0.001]. Administration of TCP resulted in marked decreases in NA levels at initial times, but the levels increased above control levels after 3.0 hr. In contrast, FTCP administration resulted in a gradual increase in NA levels (Fig. 87). Analysis of DA levels following administration of TCP and FTCP indicated a significant difference between effects of the drug treatments [F(1,78]=14.93, P<0.001], a significant effect of time [F(7,78)=19.14, P<0.001] and a significant interaction between drug treatment and time [F(7,78)=10.16, P<0.001]. After TCP administration, DA levels markedly increased above those seen after FTCP at initial times, while FTCP caused more gradual increases in DA levels above the control levels up to 24 hr (Fig. 88).

Statistical analysis of 5-HT levels after TCP and FTCP injection indicated a significant difference in effects of the two drug treatments [F(1,78)=59.62, P<0.001], a significant effect of time [F(7,78)=31.59, P<0.001] and a significant interaction between drug treatments and time [F(7,78)=15.76, P<0.001]. Administration of TCP caused more marked increases in 5-HT levels than those seen after FTCP up to the 12 hr period after administration (Fig. 89); increases in 5-HT levels appeared more slowly after treatment with FTCP.

Thus the two drugs differ in their neurochemical effects on NA, DA and 5-HT. Tranylcypromine exists as a racemate of (+) and (-)-TCP enantiomers in equal proportions. The (+)-enantiomer has been shown to be more potent than (-)-TCP at inhibiting MAO (Zirkle _et_ _al._, 1962; Fuentes, 1976; Reynolds _et_ _al._, 1980; Hampson _et_ _al._, 1986; present investigation, Table 11), whereas (-)-TCP has been demonstrated to be more effective than (+)-TCP as an inhibitor of uptake of catecholamines (Horn and Snyder, 1972). It appears that the (-) enantiomer enters the brain faster than (+)-TCP but is cleared from brain at a faster rate than (+)-TCP (Fuentes _et_ _al._, 1976; Lang _et_ _al._, 1979; Weber _et_ _al._, 1985; Hampson _et_ _al._, 1986). Thus it can be concluded that the initial effects of TCP (i.e., the ±-form) on neurotransmitters are probably due to both enantiomers, whereas neurochemical effects at later times are predominantly, if not exclusively, due to the more slowly eliminated (+)-enantiomer of TCP. The almost 2 times slower elimination rate of (+)-TCP compared to (-)-TCP (Hampson _et_ _al._, 1986) lends support to this proposal. Administration of TCP initially caused marked decreases in NA levels and this may reflect the ability of TCP to affect release and reuptake of NA at nerve terminals (Hendley and Snyder, 1968; Schildkraut, 1970; Baker _et_ _al._, 1978). McKim _et_ _al._ (1979) reported that decreases in NA levels in brain at initial times after TCP administration were accompanied by increases in levels of normetanephrine (NME), a metabolite of NA. It was proposed that both effects were due in part to the effects of TCP on NA transport at the nerve terminal. In contrast, in the present investigation FTCP did not cause an initial decrease in NA levels; this suggests differences in the relative abilities of TCP and FTCP to affect uptake and release of NA. It should be noted that in the present study FTCP was also a racemate, and that no attempt was made to separate and quantitate the individual enantiomers in tissues or blood following administration of the racemate.

Increases in DA levels after TCP injection were different from those seen after FTCP. It has been observed that TCP is a moderate releaser of DA from rat striatal slices in vitro (Baker et al., 1978) and Azzaro and Demarest (1982) reported DA uptake inhibition by TCP in a rat forebrain synaptosomal preparation; these data are consistent with the report of Hendley and Snyder (1968) on effects of TCP on catecholamine uptake systems. Since there is not an initial decrease, but rather an increase, in DA levels following TCP injection, it seems unlikely that TCP's effects on uptake and release are having much influence on the DA levels observed, and differences in the pattern of DA increases observed between the two drugs are difficult to explain at this time.

Interactions of TCP with the serotoninergic system have been studied extensively (Tuomisto and Walaszek, 1974; Green and Kelly, 1976; Holman et al., 1976; Gentil et al., 1978; Baker et al., 1980b; Smith and Petersen, 1982; Smith, 1983; Marley and Wozniak, 1983; Tuomisto and Smith, 1986). It has been reported that TCP can affect both uptake and release of 5-HT (Tuomisto and Walaszek, 1974; Baker et al., 1978), and Smith (1983) suggested that (+)-TCP predominantly influences serotoninergic while (-)-TCP influences catecholaminergic systems, based on behavioral and biochemical data. The (+)-enantiomer was more potent at potentiating T-induced convulsions than was (-)-TCP. Only after (+)-TCP injection did reserpinized, nialamide-pretreated rats show a behavioral syndrome commonly attributed to serotoninergic processes. Antagonists of 5-HT blocked this behavioural syndrome induced by (+)-TCP. Recently

Price *et al.* (1985) reported enhancement of serotoninergic function in depressed patients receiving TCP treatment, and suggested that this may be related to the therapeutic benefit. In contrast to earlier reports (Green *et al.*, 1977) suggesting nearly 80-90% inhibition of MAO-A as a prerequisite to elevating 5-HT levels, Hampson *et al.* (1986) observed significant increases in 5-HT brain levels even at 50% inhibition of MAO-A. Since *in vivo* MAO-A inhibition patterns after TCP and FTCP were similar (>85% inhibition over 24 hr period) the differences in 5-HT levels after TCP and FTCP treatments are probably not due to MAO inhibition, but possibly due to effects mentioned earlier in this thesis, such as actions on uptake and release of 5-HT, on uptake of tryptophan (the precursor of 5-HT) into the brain and/or effects on synthetic enzymes such as AADC.

During the present investigation, neurochemical effects and pharmacokinetic properties of TCP and FTCP were compared and contrasted. These studies indicated that FTCP was a more potent MAO inhibitor than TCP *in vitro*. Changes in levels of neurotransmitter amines and trace amines suggest differences in neurochemical profiles of TCP and FTCP. Higher MAO-inhibiting potency and favorable pharmacokinetic characteristics (higher, more sustained levels after administration of an equimolar dose) should enable use of FTCP at a smaller dose than TCP which would likely also result in reduced side effects. Studies on individual enantiomers would indicate possible differences between FTCP and TCP which might influence FTCP's neurochemical and pharmacokinetic profile. The pharmacokinetic studies of FTCP and TCP conducted in the present investigation also give further support to previous proposals that aromatic hydroxylation may be an important metabolic pathway for TCP in rat.

J. Pharmacokinetic and Neurochemical Studies on PGTCP

After i.p. administration of the N-propargylated compound PGTCP, peak levels in brain, blood and liver were observed at 0.5 hr (Fig. 90). Significantly higher PGTCP levels in liver than in brain suggest relatively inefficient entry *via* the blood-brain barrier. This was in contrast to a fairly similar distribution of TCP between brain and liver, and it thus appears that the introduction of a propargyl group may influence tissue distribution and transport across the blood-brain barrier. The elimination half-life (β) indicates rapid clearance from brain, blood and liver; interestingly, the β value of PGTCP was faster than that of the TCP formed from it (Table 23). Introduction of the electron-withdrawing propargyl group would render PGTCP a weaker base than TCP (a decrease in pKa) and this factor may have some influence on its clearance. Examination of AUC values (Table 23) indicates a smaller extent of N-dealkylation in liver than in brain. Interestingly, the AUC of TCP (0-12 hr) in brain was nearly 1.6 times higher than the corresponding AUC of PGTCP in brain, suggesting considerable TCP transport from the periphery (liver). In brain, blood and liver TCP was eliminated monoexponentially with a β-value of 2-3 hr; these values agree with reported values (see Section IV.I), and this was in contrast to the biphasic elimination of TCP in these tissues after administration of TCP itself. After PGTCP administration, TCP levels could not be detected beyond 12 hr (see Fig. 91), whereas after an equimolar dose of TCP itself, detectable levels of TCP were observed at 24 hr. Comparison of the availability of TCP (AUC, 0-12 hr) after administration of TCP and PGTCP (each at a dose of 0.1 mmol/kg, i.p.) indicated that TCP administration itself caused nearly 14, 26 and 10 times more TCP available in blood, brain and liver respectively than did the administration of PGTCP. This would suggest that PGTCP did not improve the elimination characteristics of TCP. Three possible explanations could be put forward for the lack of sustained TCP levels after administration of PGTCP:

1. The prodrug itself was eliminated at a faster rate than TCP and hence could not achieve the desired effect in any tissue.
2. Unfavorable transport characteristics across the blood-brain barrier did not permit elevated prodrug levels in brain.
3. It is likely that PGTCP might undergo other metabolic reactions, such as aromatic hydroxylation, which, if they exceeded N-depropargylation, would also result in decreased levels of PGTCP and TCP.

Levels of the trace amine PEA were measured in blood, brain and liver after injection of PGTCP. Levels of PEA in blood after PGTCP treatment were not different from control values and this was in contrast to marked changes in blood PEA levels after administration of TCP. A comparison of PEA levels in brain, blood and liver with those seen after an equimolar dose of PGTCP (Figs. 82, 83, 84 and 92) indicates that PGTCP treatment was not as effective as TCP treatment in increasing PEA levels. In contrast to the nearly 25-30 fold increases in PEA levels after TCP treatment, PGTCP treatment caused only 8-15 fold increases in the brain and liver.

The *in vitro* MAO inhibition data indicate that PGTCP was a weaker inhibitor of MAO-A and -B (about 90 fold) than TCP (Table 18). Introduction of the N-propargyl group onto N-methylamphetamine resulted in formation of a potent MAO inhibitor (deprenyl), whereas the same functional group on TCP rendered PGTCP a weaker MAO inhibitor than TCP itself. It is conceivable that the N-propargyl group on TCP might hinder optimum interaction of TCP with the active site of MAO for effective MAO inhibition. In vivo inhibition data indicate nearly 80% inhibition of MAO in brain up to 24 hr after PGTCP administration (Table 23). In vitro data did not predict this in vivo potency, but it is probably due primarily to the TCP formed in this situation. Although TCP levels in brain were lower than after administration of TCP alone, the levels obtained would be similar to those expected after administration of 1-2 mg/kg TCP. Recent studies (Hampson et al., 1986a) have demonstrated that low dose TCP administration can cause marked MAO inhibition; these workers observed nearly 90% inhibition of MAO-A and -B in brain 1.5 hr after administration of TCP (1 mg/kg, i.p.). In addition, metabolites of PGTCP other than TCP might arise due to aromatic hydroxylation or aromatic hydroxylation followed by N-depropargylation and contribute to the in vivo MAO inhibitory profile.

Despite relatively marked MAO inhibition, the levels of PEA were not increased proportionately. One possible explanation might be that the residual MAO activity is sufficient enough to cause considerable metabolism of PEA, an excellent substrate with high affinity for MAO-B. Alternatively, the observed MAO inhibition might be restricted only to an MAO pool with a slow turnover, leaving the MAO pool with the faster turnover rate relatively unaffected. The existence of such pools was proposed by Maitre et al. (1976). Changes in the levels of precursor amino acid and the activity of synthetic enzymes such as AADC might also influence PEA levels in brain.

Levels of NA, DA and 5-HT after administration of PGTCP were determined by HPLC-EC (Fig. 93). The increases in these biogenic amines were similar to those seen after TCP administration although there were some differences. Analysis of NA levels (0-24 hr) after TCP and PGTCP treatments (Figs. 87 and 93) indicated a significant difference between effects of treatments [F(1,71)=61.84, P<0.001], a significant effect of time [F(7,71)=95.61, P<0.001] and a significant interaction between drug treatment and time [F(7,71)=8.26, P<0.001]. Similar analyses of DA levels (Figs. 88 and 93) indicated a significant difference between effects of treatments, a signficant effect of time and a significant interaction between treatment and time [F(1,76)=174.14, P<0.001; F(7,76)=21.00, P<0.001 and F(7,76)=14.60, P<0.001 respectively]. Equivalent analyses of 5-HT (Figs. 89 and 93)levels also indicated a significant difference between effects of treatment, a significant effect of time and a significant interaction between drug treatment and time [F(1,77)=123.37, P<0.001; F(7,77)=88.21, P<0.001 and F(7,77)=17.43, P<0.001 respectively].

In contrast to the marked decreases in NA levels followed by increases seen after TCP treatment, PGTCP treatment resulted in gradual increases in NA levels. These differences are probably due to differences between TCP and PGTCP in affecting uptake and release of NA in nerve terminals. Administration of PGTCP caused relatively smaller increases in DA levels relative to increases observed in NA and 5-HT. Levels of DA at 0.5 hr were significantly lower than control levels, perhaps indicating release of DA by PGTCP. Green _et al._ (1977) proposed that inhibition of MAO-A and -B of at least 80% was essential to increase DA and 5-HT functional activity; despite such inhibition observed in the present study, brain DA levels were not markedly increased. This raises a serious question as to the role of MAO inhibition in the increase in functional activity of biogenic amines. Philips and Boulton (1979) conducted a comprehensive time-dose study on a number of MAO inhibitors and despite the nearly 100% inhibition of MAO-A and MAO-B expected at the high doses employed, many of the drugs tested produced quite different patterns in increases of trace amine levels. No such comparative studies were conducted with the classical neurotransmitter amines, although it is likely that a similar situation also exists for these classes of biogenic amines. This would suggest that in future studies on MAO inhibitors, the role of MAO inhibition should be considered along with effects on uptake and release, availability of precursors for the amines, activity of synthetic (and other degradative) enzymes as well as levels and metabolism of the drug concerned. The relative increases in 5-HT levels were higher than those seen for NA and DA. This is similar to effects that were observed after administration of TCP itself in the present study (Fig. 89) and those reported by others after chronic administration of high (Baker et al., 1984c) and low (Hampson et al., 1986a) doses of TCP. It is also worthy of mention at this time that at any given time two enantiomers of TCP and PGTCP would be present in brain and the observed neurochemical changes are conceivably due to interactions of these four isomers with the neurotransmitter systems.

It would be advantageous to have MAO inhibition localized to brain in order to minimise unpleasant side effects due to peripheral MAO inhibition, and currently such MAO inhibitors are being developed (see Section I.G). Preferential localization of PGTCP in periphery and the relatively small extent of biotransformation to TCP would indicate that either the N-propargyl group on TCP was metabolically relatively nonlabile or conferred upon the drug unfavorable physicochemical properties. However, PGTCP may present an useful TCP analog with which to study metabolism and dispositon of N-alkylated cyclopropylamines.

K. Analysis of PLZ in Rat Brain and Patient Urine Samples by Extractive Pentafluorobenzoylation

Phenelzine is a hydrazine-type MAO-inhibiting antidepressant which has proved to be useful in the treatment of nonendogenous depression and phobic disorders (Robinson *et al.*, 1978). Despite its widespread therapeutic use, few studies have been conducted on the pharmacokinetics and metabolism of this drug in brain tissue. Recent investigations in the brain (Baker *et al.*, 1982; Dyck *et al.*, 1985) indicate that PLZ undergoes metabolism to PEA, the functional deficiency of which has been implicated in the etiology of depressive disorders. It has been reported that PLZ can alter the uptake and release of putative neurotransmitter amines in brain tissue (Hendley and Snyder, 1968; Baker *et al.*, 1978, 1980b; Dyck, 1983, 1984), and because of this it is important to know the concentrations of the drug in the brain.

There is a paucity of reports on PLZ analysis in the literature. One gas chromatographic method involved formation of the hydrazone derivative with acetone under alkaline conditions and extraction of the derivative followed by GC analysis (Caddy *et al.*, 1976). The procedure was not applied to brain samples. Cooper *et al.* (1978), in a study on PLZ in plasma, have used extraction into an organic solvent mixture at pH 6.8 followed by derivatization with heptafluorobutyric anhydride under anhydrous conditions and reported picomole sensitivity. The method is rather lengthy and was not used for analysis in brain tissue. Jindal et al. (1980) have used pentafluorobenzaldehyde to derivatize PLZ under aqueous conditions (pH 6.7), extracted the derivative into organic solvent, taken the extract to dryness and quantitated the derivative by GC-MS with single ion monitoring (GC-MS-SIM); a sensitivity of 2 ng/ml was attained. Dyck (1984) has reported the use of combined TLC-MS-SIM assay methodology to quantitate PLZ as its dansylated acetonephenylethylhydrazone derivative in rat tissue samples. Such mass spectrometric techniques provide a high degree of specificity, but are lengthy and there are high costs involved with equipment purchase and maintenance.

Caddy et al. (1976) reported that extraction of PLZ into organic solvents from basified aqueous solutions was relatively poor, thus necessitating extraction and derivatization to be combined in one single step. In the present investigation, PLZ analysis was performed by aqueous pentafluorobenzoylation which similarly combines extraction and derivatization in a single step and affords a simple, rapid and sensitive analytical technique.

The use of pentafluorobenzoylation under aqueous conditions means that derivatization and extraction are combined, which saves time and also provides derivatization under relatively mild conditions.

Phenelzine is reported to be susceptible to auto-oxidation and decomposition under alkaline conditions (Eberson et al., 1962; Schlitt et al., 1967). However, during the development of the present assay methodology a wide range of alkaline pH values was studied and no evidence of instability was observed under the conditions described in the Section II.D.3.

A hydrazine such as benzylhydrazine might be considered a more appropriate internal standard than CPEA to use for an assay for PLZ. However, benzylhydrazine, like PLZ, forms two derivatives, and this presents the problem of adequately separating all four derivatives. Derivatized 2-(4-chlorophenyl)-ethylamine (CPEA) separated readily from both derivatives of PLZ and provided a linear calibration curve and was therefore adopted as the internal standard.

Dyck (1984), utilizing a mass spectrometric procedure, reported levels of PLZ of 500 ng/g in rat striatum 2 hr after injection of a dose of PLZ equivalent to approximately 60 mg of free base/kg. The whole brain levels of PLZ in the present study (Section III.K) were in close agreement with her results. In 13 patients receiving 15 mg of PLZ sulfate t.i.d. for 2 weeks, the mean excretion of PLZ in 24 hr urine samples was 1211 ± 356 (mean ± S.E.M.) μg. This appears to be in reasonable agreement with the findings of Johnstone (1976) and Caddy _et al._ (1976) who reported values of 698 ± 124 (n=15) and 1575 ± 299 (n=12) μg/24 hr in fast and slow acetylators respectively after these patients had been receiving 30 mg phenelzine t.i.d. for 13 days. The patients studied in the present investigation were not divided into slow and fast acetylators.

In summary, a novel, rapid and relatively inexpensive analytical procedure was developed for studying neurochemical and pharmacokinetic properties of PLZ. Although the development of this assay did not represent a major portion of the study reported in this thesis, it did serve to demonstrate the utility of one of the assay techniques (in this case aqueous pentafluorobenzoylation) developed during the course of the study and provided a technique for analysis of an important drug which had previously proven difficult to measure.

V. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions result from the studies described above:

1. Administration of CPPEA, CEPEA, PGPEA or DPGPEA results in sustained elevations of brain PEA levels, indicating that these N-alkylated analogs act as prodrugs *in vivo*.
2. Based on their abilities to cause elevated, sustained levels of PEA in brain, the order of effectiveness of the prodrugs is PGPEA>DPGPEA>CEPEA>CPPEA.
3. Unlike MAO inhibitors, the PEA prodrugs elevate PEA without causing dramatic increases in brain levels of T, the catecholamines (NA and DA) and 5-HT, although all four drugs do cause some significant, but transient, changes in brain levels of the catecholamines, 5-HT and their metabolites; these effects are most marked with CPPEA.
4. The N-propargyl analogs of PEA (PGPEA and DPGPEA) are weak inhibitors of MAO *in vitro*, with moderate selectivity towards MAO-B. Both drugs also show a preference for inhibition of MAO-B *in vivo*, and this selectivity is more marked in liver than in brain. The dipropargyl compound DPGPEA is actually a double prodrug, being converted to both PGPEA and PEA.
5. Tryptamine may undergo a significant first-pass metabolism in the periphery since intraperitoneal administration results in only moderate increases in brain T levels.
6. Administration of CET causes elevated, sustained levels of T but CET displays some disadvantages as a prodrug of T.
7. A number of structural analogs of TCP, namely TTCP, MDTCP, MTCP and NCP, are equipotent to or more potent than TCP itself at inhibiting MAO *in vitro*.

8. The ring-fluorinated compound FTCP is 10 times more potent as a MAO inhibitor than TCP _in vitro_ and exhibits more favorable pharmacokinetic properties _in vivo_ than TCP. Aromatic hydroxylation may be an important metabolic pathway for TCP. At the dose studied, FTCP and TCP do not differ in their _in vivo_ MAO inhibiting potencies, although they differ in their neurochemical effects on levels of trace amines and neurotransmitter amines in brain.
9. The propargyl analog PGTCP is a weaker MAO inhibitor _in vitro_ than TCP. Biotransformation to TCP and/or other potent MAO inhibitors is probably responsible for the relatively strong MAO inhibition observed _in vivo_ after administration of PGTCP. Since PGTCP exhibits marked peripheral tissue localization, rapid clearance and limited biotransformation to TCP, it may not represent a good prodrug of TCP, but might be of help in designing better prodrugs of TCP in future.
10. Aqueous pentafluorobenzoylation and aqueous pentafluorobenzenesulfonylation followed by GC-ECD analysis are extremely useful techniques which have provided for analysis of PEA, TCP, T and their analogs under investigation in this study but which should also have application to analysis of a wide variety of bioactive amines, including drugs.

During the course of the investigations that are described in this thesis, it became apparent that much of this research should be extended in the future. In my opinion, future research along the following lines would do much to increase our understanding of the mechanisms of action of the prodrugs of amines and of the TCP analogs:

1. In order to learn more about the localization of the prodrugs in brain and possible locations of their metabolism, it would be advisable to dissect out discrete brain areas and also to prepare subcellular fractions from brain areas and to investigate the concentrations of both the prodrug and the amine formed from it in those areas and in the subcellular fractions.
2. During the course of the present investigation administration of prodrugs of trace amines was shown to alter levels of neurotransmitter amines and their metabolites. It is possible that the prodrugs influence the uptake and release of these neurotransmitters as other phenylalkylamines have been reported to do (Koe, 1976; Baker, 1980a; Suckling, 1985). It is now necessary to study effects of the prodrugs on in vitro uptake and release of neurotransmitters alone and in combination with the respective trace amines. Such experimental data would be of help in delineating neurochemical effects of prodrugs from the trace amines formed from them. These studies, coupled with in vivo measurements of the O-methylated metabolites NME, MHPG and 3-methoxytyramine would give further information about the effects of the prodrugs (and amines formed from them) on turnover and thus help characterize the nature of the interaction with the neurotransmitter amines.
3. Chronic studies should now be carried out in which the prodrugs or TCP analogs are administered over a 2-4 week period (the time required for antidepressants to show efficacy in the clinical situation). Such studies should include monitoring during this period of a behavioral component known to be related to antidepressant activity, and at the end of the period the brain tissue should

be analyzed for levels of the prodrug or analog, the amine of interest, and levels of neurotransmitter amines and their metabolites. It would also be of interest to carry out similar chronic experiments in other sets of animals and to study in them changes in number and affinity of adrenergic and serotoninergic receptors since several antidepressants have been reported to alter sensitivity of subclasses of these receptors after chronic, but not acute, administration (review: Blier and DeMontigny, 1985).

4. In addition to FTCP, several other analogs of TCP displayed potent MAO-inhibitory properties. It would seem worthwhile to conduct pharmacokinetic and neurochemical studies on these drugs as well. Because the findings on FTCP were so promising, it would be important to carry out comprehensive dose-response studies on its MAO-inhibiting properties _in vivo_ to determine the minimum dose at which 85% inhibiton of brain MAO is obtained; this is a level of inhibition at which it has been proposed there is a general elevation of brain monoamines (Ravaris _et al._, 1976) and is also a level (based on platelet MAO activity) at which there is clinical improvement in a high percentage of patients (Robinson _et al._, 1978). Such studies are now being carried out in the Neurochemical Research Unit by others and will be followed by investigations of chronic administration of this drug at that dosage level.

5. Tranylcypromine, FTCP and the other analogs of TCP are racemates, and it would be of interest to study in brain the levels of the separate enantiomers after acute and chronic administration of the racemic drugs. This is an area of biological psychiatry that has been much neglected despite the fact that many psychiatric drugs

are racemic in nature. The marked differences in pharmacological and neurochemical properties of the individual enantiomers of TCP reported by other researchers (see Introduction of this thesis) certainly emphasize the importance of performing analysis on the separate isomers. Separation and analysis of these enantiomers can be achieved using chiral capillary columns or chiral derivatizing agents and GC and should prove to be an exciting area of research.

6. Future studies on the prodrugs and the TCP analogs should also include an investigation in heart of the levels of the drugs, the bioactive amines formed from them (in the case of prodrugs), and potentially active metabolites (such as ring hydroxylated metabolites). Tranylcypromine is known to cause some side effects which involve the cardiovascular system (Baldessarini, 1980), and PEA and T, like TA, can cause release of putative neurotransmitter amines from cardiac tissue (Paton, 1976). Recent studies in our laboratories have shown that the levels of CETCP, (a prodrug of TCP), TCP and one of its metabolites, HTCP, are markedly different in heart and brain (Nazarali _et al._, 1986c) following intraperitoneal injection of the prodrug, and it appears that further studies in this area are certainly warranted.

In summary, the investigations described in this thesis have demonstrated that several N-alkylated analogs of PEA can serve as useful prodrugs with which to attain elevated and relatively consistent and sustained levels of PEA in brain. When these drugs are administered on a chronic basis and combined with behavioral experiments, they should yield important information about the role of PEA in the CNS and in the action of antidepressant drugs. It is also conceivable that some of these prodrugs might be effective antidepressants, particularly if there is a functional deficiency of PEA in certain subtypes of depression. In any case, it certainly appears that they will be effective pharmacological tools with which to study prodrug metabolism. The 4-fluoro derivative of TCP studied in these investigations has stronger MAO-inhibiting properties than TCP and also appears to have more favorable pharmacokinetic properties than TCP; this drug is currently being investigated further in combined behavioral/neurochemical studies in the Neurochemical Research Unit. Several other TCP analogs studied also had strong MAO-inhibiting properties *in vitro* and will be investigated further by other researchers in the Unit. Studies of the pharmacokinetics of the prodrugs and TCP analogs were made possible by the development of sensitive electron-capture gas chromatographic procedures, and this became an important component of the research described in this thesis. Such analyses will be used or modified by others who will extend this work.

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